U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-281 727

Soluble Organic Nitrogen Characteristics and Removal

Stanford Univ, Calif Dept of Civil Engineering

Prepared for

Municipal Environmental Research Lab, Cincinnati, Ohio Wastewater Research Div

Mar 78

PB 281 727

EPA-600/2-78-030
March 1978

Environmental Protection Technology Series

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ENVIRONMENTAL PROTECTION TECHNOLOGY series. This series describes research performed to develop and demonstrate instrumentation, equipment, and methodology to repair or prevent environmental degradation from point and non-point sources of pollution. This work provides the new or improved technology required for the control and treatment of pollution sources to meet environmental quality standards.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/2-78-030	2.	3. RECIPIENT'S ACCESSION NO. PB281727

4. TITLE AND SUBTITLE SOLUBLE ORGANIC NITROGEN CHARACTERISTICS AND REMOVAL	5. REPORT DATE March 1978 (Issuing Date)
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Stephen J. Randtke, Gene F. Parkin, John V. Keller, James O. Leckie, and Perry L. McCarty	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Stanford University Stanford, California 94305	10. PROGRAM ELEMENT NO. 1BC611 - SOS #3 - Task 32
	11. CONTRACT/GRANT NO. R-804001
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory--Cin.,OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268	13. TYPE OF REPORT AND PERIOD COVERED Final - Sept. '74 - Feb. '77
	14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES

C. Mashni, Project Officer
U.S. EPA - Cincinnati, Ohio 45268 513/684-7684

16. ABSTRACT

This report discusses sources, concentrations, characteristics and methods for removal of Soluble Organic Nitrogen (SON) in wastewater. Removal by various physical, chemical and biological processes are described and molecular weight distribution is characterized. A significant portion of the SON in secondary effluent is produced biologically during treatment.

Chemical coagulation, ion exchange and activated carbon were used singly and in combination to characterize different fractions of the SON and the Soluble Chemical Oxygen Demand (SCOD).

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
wastewater activated sludge nitrification chemical removal (sewage treatment) nitrogen	soluble organic nitrogen, soluble chemical oxygen demand, molecular weight distribution, effluent standards, sorption coagulation, biodegradation	13B

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 274
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE PC A12 MF A01

EPA Form 2220-1 (9-73)

☆ U. S. GOVERNMENT PRINTING OFFICE 1978-757-140/6807 Region No. 5-11

EPA-600/2-78-030
March 1978

SOLUBLE ORGANIC NITROGEN CHARACTERISTICS AND REMOVAL

by

Stephen J. Randtke, Gene F. Parkin, John V. Keller,
James O. Leckie, and Perry L. McCarty
Department of Civil Engineering
Stanford University
Stanford, California 94305

Grant No. R-804001

Project Officer

Charles I. Mashni
Wastewater Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

1A

DISCLAIMER

This report has been reviewed by the Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. The complexity of the environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

Characterization of wastewater components and identification of their sources is imperative in our continuing search for new and improved technologies for pollution abatement. This publication provides much needed information on the sources, treatability and fate of the nitrogen containing organic compounds present in wastewater.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

ABSTRACT

Soluble organic materials containing nitrogen (SON) are present in effluents from activated sludge treatment of municipal wastes. The objective of this study was to determine the sources, concentrations, characteristics, and methods for removal of SON to aid in the establishment of possible regulatory criteria and in the design of treatment systems for its control.

SON ranged from 1.1 to 2.1 mg/l in seven composite effluent samples taken from four conventionally operated activated sludge plants which varied in size, location, and influent waste characteristics. About two-thirds of the SON in such effluents is estimated to be residual from the untreated waste and the remainder is produced biologically during treatment.

About one-half of the biologically produced SON results from organism decay, the concentration depending upon detention time and mixed liquor suspended solids concentration. The other one-half represents excreted materials, which approach a concentration in equilibrium with living cells that is independent of cell concentration or aeration time. These biologically produced materials are relatively non-biodegradable. During process start-up or stress, biologically produced SON may reach several mg/l, but this excess material is quite biodegradable.

Ozone, chlorine (breakpoint), permanganate, and peroxide removed an average of 14 ± 7, 42 ± 4, 28 ± 3, and 19 ± 3 percent of the SON, respectively, and 46 ± 4, 18 ± 7, 0 ± 2, and 2 ± 2 percent of the soluble chemical oxygen demand (SCOD), respectively. Ozone and chlorine removed different fractions of the SON. Preozonation increased the fraction of SON removable by chlorination by about 30 percent.

Chemical coagulation, ion exchange, and activated-carbon adsorption were used singly and in combination to characterize different fractions of the SON and SCOD. About 10 percent of the SON was removed by cation exchange and coagulation at high pH, indicating that it was positively charged; this fraction was not removed by activated carbon. About 20 percent of the SON appeared to be negatively charged since it was removed by coagulation at pH 6 but not at pH 10. Activated carbon removed a relatively non-polar 70 percent of the SON. Various coagulants specifically adsorbed 0 to 20 percent of the SON, entrapped 5 percent with a larger size, and electrostatically adsorbed the positively and negatively charged fractions. Ten percent of the SON removed by anion exchange was also removed by activated carbon. A scheme is provided which divides SON and SCOD into a number of such fractions, indicating which are mutually exclusive and which overlap.

Less than 10 percent of SON in secondary effluents consists of individual or combinations of amino acids. Approximately 25 to 35 percent appear to be heterocyclic compounds, probably nucleic acid degradation products having apparent molecular weights of less than 780, similar to that in untreated municipal wastewater. The excess SON produced during start-up, however, generally had a much higher molecular weight distribution.

This report was submitted in fulfillment of Research Grant No. EPA-R-804001 by Stanford University under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from September 1, 1974 to May 1, 1977, and work was completed as of June 15, 1977.

CONTENTS

Foreword iii
Abstract iv
Figures viii
Tables x
List of Abbreviations and Symbols xiv
Acknowledgements xvi

1. Introduction 1
2. Conclusions 3
3. Recommendations 7
4. Sources, Occurrence, and Chemical Nature of Organic Nitrogen . . 8
5. Materials and Methods 17
6. SON in Municipal Secondary Effluents 21
7. SON Removal by Physical and Chemical Processes 33
    Removal Mechanisms 33
    Experimental Approach 36
    Chemical Coagulation 41
    Ion Exchange and Activated-Carbon Adsorption 54
    Chemical Oxidation 68
    Processes in Sequence 79
    Discussion of Results 91
8. SON Formation and Removal by Biological Processes 103
    Introduction 103
    Nomenclature 103
    Biologically Produced SON 104
    Refractory Organics 104
    SON Production and Excretion by Bacteria 110
    Summary and Basis for Experimental Approach 114
    Laboratory Activated Sludge Cultures 115
    Batch Experiments 117
    SON Removal 119
    SON Production 130
    Discussion of Results 169
9. SON Characterization 186
    Introduction 186
    Results 189

Preceding page blank

9. SON Characterization (continued)
    Discussion of Results 206

10. Summary and Discussion 212
    SON in Municipal Secondary Effluents 212
    SON Characteristics 212
    Control of SON 214
    Ecological Significance of SON 215

References 217

Appendices

A. Statistical Calculations 234

B. Nitrate Interference with Kjeldahl SON Analysis 251

FIGURES

Number Page

1 Diurnal Variation of SON--Palo Alto (4-14-75) 27

2 Diurnal Variation of SON--San Jose/Santa Clara (5-13-75) 28

3 Diurnal Variation of SON--Union City #3 (5-1-75). 29

4 Diurnal Variation of SON at Union City #3 (5-1-75) 30

5 Removal of SON by Ion Exchange as a Function of pH 61

6 Removal of SCOD by Ion Exchange as a Function of pH 62

7 Operational Fractionation of SON and SCOD 100

8 Laboratory Activated-Sludge System 116

9 Batch Study 1: Effect of Aeration Time on SON Remaining, $MLSS_{om} \simeq 1300$ mg/l 121

10 Batch Study 1: Effect of Aeration Time on SCOD Remaining, $MLSS_{om} \simeq 1300$ mg/l 122

11 Batch Study 2: Effect of Sludge Washing and Aeration Time on SON Removal from unfiltered primary effluent, $MLSS_{om} = 1200$ mg/l. 123

12 Batch Study 3: Effect of $MLSS_{om}$ on SON Removal from Filtered Primary Effluent 125

13 Batch Study 3: Effect of $MLSS_{om}$ on SCOD Removal from Filtered Primary Effluent 126

14 Batch Study 4: Effect of Substrate on SON Remaining, $MLSS_{om} \simeq 180$ mg/l 128

15 Batch Study 4: Effect of Substrate on SCOD Remaining, $MLSS_{om} \simeq 180$ mg/l 129

16 AS Culture 1 Start-Up: SON and MLSS Concentrations vs Time of Operation 131

17 AS Culture 2: SON and MLSS Concentrations vs Time of Operation (All Data) 132

18 AS Culture 1, Days 20-340: SON and MLSS Concentrations vs Time of Operation 135

19 Initial Release of SON for AS Culture 1 as a Function of MLSS Concentration 139

20 Initial Release of SCOD for AS Culture 1 as a Function of MLSS Concentration 140

Number | Page

21 The Effect of Aeration Time on SON and SCOD Release during Organism Decay at Different MLSS Values Using AS Culture 1 (Day 274) 147

22 The Effect of Aeration Time on SON Release during Organism Decay at Different MLSS Concentrations Using Palo Alto Activated Sludge (9-9-75) 148

23 The Effect of Aeration Time on SCOD Release During Organism Decay at Different MLSS Concentrations Using Palo Alto Activated Sludge (9-9-75) 149

24 The Effect of MLSS Concentration on SON and SCOD Release during Organism Decay of AS Culture 1 152

25 Batch Study 5: Effect of SCOD_{oc} on SON Production Using AS Culture 2, $\text{MLSS}_{om} \approx 580$ mg/l (Day 171) 157

26 Batch Study 6: Effect of SCOD_{oc} on SON Production Using AS Culture 1 Sludge, $\text{MLSS}_{om} \approx 216$ mg/l (Day 61) 158

27 SCOD Removal vs Aeration Time in Batch Study 6. $\text{MLSS}_{om} \approx$ 216 mg/l (Day 61) 159

28 Batch Study 7: Effect of Substrate Type on SON Production Using AS Culture 1, $\text{MLSS}_{om} \approx 360$ mg/l (Day 98) 162

29 Batch Study 7: SCOD Removal vs Aeration Time, $\text{MLSS}_{om} \approx 360$ mg/l (Day 98) 163

30 Batch Study 8: Effect of MLSS_{om} on SON Production 164

31 Batch Study 9: Effect of NH_3-N on SON Production, MLSS ≈ 830 mg/l (Day 176) 165

32 Conceptual Model of Changes in SON_p, SON_g, SON_d, and SON_{eq} with Batch Activated-Sludge Aeration Time 171

33 Conceptual Model of Changes in Sources of Effluent SON as a Function of Activated-Sludge Aeration Time 179

34 Molecular Weight Distribution of Recovered SON and SCOD for Soluble Raw Wastewater as Percent of Mass in Original Sample. ± Figures are 95 percent confidence limits 199

35 Molecular Weight Distribution of Recovered SON and SCOD for Soluble Primary Effluent as Percent of Mass in Original Sample. ± Figures are 95 percent confidence limits 200

36 Molecular Weight Distribution of Recovered SON and SCOD for Soluble Activated-Sludge Effluent as Percent of Mass in Original Sample. ± Figures are 95 percent confidence limits 201

37 Molecular Weight Distribution of Recovered SON and SCOD for Soluble AS Culture 2 Effluent during Start-Up as Percent of Mass in Original Sample. ± Figures are 95 percent confidence limits. . 202

TABLES

Number		Page
1	Sources of Organic Nitrogen in Municipal Wastewater	9
2	Percentage of Total Organics in the Soluble Fraction of Raw Wastewater	10
3	Nitrogen Forms in Two Raw Wastewaters	11
4	Non-Amino Acid Nitrogenous Constituents of Domestic Wastewater	11
5	Total and Soluble Organics in Secondary Effluent	13
6	Characterization of Soluble Secondary Effluent Organics	14
7	Precision of SON and SCOD Analyses	18
8	Significant Differences in SON and SCOD Analyses	19
9	Effect of Initial Ammonia Distillation pH on the SON Analysis	19
10	General Characteristics of the Influent and Primary Effluent Wastewaters	23
11	General Characteristics of the Secondary Effluents	24
12	Summary of SON Concentrations at Secondary Treatment Plants	25
13	General Characteristics of Palo Alto Secondary Effluent	31
14	Removal Mechanisms and Factors Affecting Removal	33
15	Treatment Processes and Removal Mechanisms	35
16	Comparison between Filtered and Unfiltered Coagulated PASE Samples	41
17	Coagulation of Filtered and Unfiltered PASE Samples	42
18	SON Removal as a Function of Mixing Time at 25 rpm	43
19	Effect of pH on Ferric Chloride Coagulation of SON	43
20	SON and SCOD Removal by Ferric Chloride Coagulation	45
21	SON and SCOD Removal by Alum Coagulation	46
22	SON and SCOD Removal by Lime Coagulation	47
23	Comparison of SON and SCOD Removal by Ferric Chloride, Lime, and Alum Coagulation	48
24	Effect of Sequential Coagulation on SON Removal	50
25	Additional SON Removals Achieved by Sequential Coagulation	51

Number Page

26 Removal of SON and SCOD by Addition of Ferric Chloride and C-31 Coagulant to Activated-Sludge 53

27 Manufacturers' Specifications for the Ion-Exchange Resins Used . 57

28 Effect of Sequential Contacting and Dosage of Ion-Exchange Resins on Removals from PASE 58

29 SON and SCOD of Deionized Water Samples Contacted with Ion-Exchange Resins and Activated Carbon 59

30 Removal of SON and SCOD by Sequential Contacting with Ion-Exchange Resins 63

31 Removal of SON and SCOD by Activated Carbon and Ion-Exchange Resins 64

32 Removal of SON and SCOD from Treated PASE at Neutral pH 65

33 Removal of SON and SCOD by Activated Carbon as a Function of pH . 65

34 Summary of Activated-Carbon Adsorption Data at Neutral pH 66

35 Summary of SON and SCOD Removal by Ion Exchange 67

36 Removal of SON and SCOD by Chlorine, Permanganate, and Peroxide . 74

37 Effect of Dechlorination Method of Lawrence *et al*. 75

38 Removal of SON and SCOD by Breakpoint Chlorination 76

39 Removal of SON and SCOD by Ozonation 77

40 Comparison of SON and SCOD Removals by Chlorine and Ozone 78

41 Removal of SON and SCOD by Combinations of Chlorine and Ozone . . 80

42 Removal of SON and SCOD by the South Tahoe P.U.D. Water Reclamation Plant 82

43 Results of Laboratory Advanced Wastewater Treatment 83

44 Removal of SON and SCOD by Ozone in Sequence with Chemical Coagulation 85

45 Removal of SON and SCOD by Ozone in Sequence with Ion Exchange and Activated-Carbon Adsorption 86

46 Removal of SON and SCOD by Ozonation and Activated-Carbon Column Adsorption 87

47 Removal of SON and SCOD by Ferric Chloride Coagulation and Ion Exchange 88

48 Removal of SON and SCOD from Coagulated PASE by Ion Exchange at Neutral pH 89

49 Removal of SON and SCOD by Coagulation, Ion Exchange, and Activated-Carbon Adsorption 90

50 Summary of Molecular Characteristics Affecting Removal of Organic Contaminants 92

Number Page

51 Summary of Removals Achieved by Individual Processes 94

52 Summary of Results of Chemical Oxidation Experiments 101

53 Biologically Produced SON Compounds 105

54 Typical Nucleic Acid Degradation Products 109

55 Composition of Synthetic Waste 117

56 Filtration and SON Analysis Used for Batch Studies 118

57 Summary of Substrates, Filtration, and SON Analysis Used during Removal Studies 119

58 The Effect of Growth Rate on AS Culture 2 Effluent SON during Culture Start-Up 133

59 Summary of Steady-State Data for Activated-Sludge Cultures . . . 134

60 Characteristics of Mixed Liquor Suspended Solids for Activated-Sludge Cultures 136

61 Effect of Culture on SON_q and $SCOD_q$ 141

62 SON Released by Sequential Dilution of Palo Alto Activated Sludge 141

63 Initial Release of SON and SCOD upon Exposure to Tap Water, AS Culture 1 Filtrate, and Released Organics 143

64 Effect of Temperature on Initial Release of SON and SCOD 144

65 Initial Release of SON and SCOD: Comparison of Substrates . . . 145

66 Equilibrium SON and SCOD Values for the Cultures Studies 146

67 Summary of Regression Analysis Coefficients Correlating SON and SCOD Release with Aeration Time 150

68 Linear Regression Equations Showing the Effect of MLSS on SON and SCOD Release during Organism Decay of Palo Alto Activated Sludge. 153

69 Variables Studied and Filtration and SON Techniques Used during Synthetic Feed Studies 154

70 Summary of SON_q Data for All Experiments 167

71 Summary of Data Describing SON Peaks during Aeration 168

72 Contribution of SON_{eq}, SON_d, and SON_g to SON_p for AS Cultures . . 176

73 Estimation of SON_{eq}, SON_d + SON_g, SON_r, and SON_b in Palo Alto Secondary Effluent (MLSS = 1390 mg/l) 182

74 Estimation of SON_{eq}, SON_d + SON_g, SON_r, and SON_b in Palo Alto Secondary Effluent (MLSS ≃ 180 mg/l) 183

75 Estimation of SON_{eq}, SON_d + SON_g, SON_r, and SON_b in Palo Alto Secondary Effluent at t_{min} 184

76 List of Samples Characterized 186

Number Page

77 Compounds Used for Molecular Weight Calibration of Sephadex Columns 188

78 Wastewaters Evaluated for Biodegradability 189

79 Summary of Rate and Extent of Biodegradability for Treated and Untreated Wastewaters 190

80 Biodegradability of Remaining Soluble Organics after Various Periods of Treatment of Primary Effluents 191

81 Summary of Rate and Extent of Biodegradability for Biologically Produced Organics 193

82 Summary of SON and SCOD Recoveries from Concentration and Filtration 196

83 Summary of SON Elution Data for Molecular Weight Distribution Studies 197

84 Summary of SCOD Elution Data for Molecular Weight Distribution Studies 198

85 SON/SCOD Ratios for the Molecular Weight Fractions of the Filtered Wastewaters Studied 204

86 Theoretical SON/SCOD for Typical Nitrogen-Containing Organic Compounds of Differing Molecular Weights 205

87 Amino Acid Data for Palo Alto Activated-Sludge Effluent (6-14-74) 207

88 Summary of Biodegradability Data 208

89 Comparison of Results from MW Distribution and Cationic Exchange Studies 210

A.1 Confidence Intervals for SON and SCOD Analyses 238

A.2 Significant Differences in Percent Removal 240

A.3 Removal of SCOD be Ferric Chloride and Lime 240

A.4 Precision and Accuracy of Low-Level Kjeldahl SON Analyses 242

A.5 Precision and Accuracy of Technicon SON Analysis 243

A.6 Precision of Low-Level SCOD Analysis 244

A.7 Precision of Kjeldahl SON and SCOD Analyses at High Concentrations 244

A.8 Confidence Intervals for SON and SCOD Analysis 246

A.9 Confidence Intervals for Differences Involving SON_{oc} and $SCOD_{oc}$. 246

B.1 Effect of NO_3^--N Concentration on SON Recovery from a Biologically Treated Wastewater 252

B.2 Effect of NO_3^--N Concentration on SON Recovery from Palo Alto Activated-Sludge Effluent 252

B.3 Effect of Compounds Tried for Elimination of Nitrate Interference 253

LIST OF ABBREVIATIONS AND SYMBOLS

A -- fitting parameter in first-order decay model

a -- linear regression coefficient (y-axis intercept)

b -- linear regression coefficient (slope)

$dSCOD_{min}$ -- minimum SCOD concentration observed during a batch experiment; obtained by subtracting the initially calculated SCOD ($SCOD_{oc}$) from the minimum SCOD measured ($SCOD_{min}$) during the experiment, mg/l

$dSON_{pk}$ -- maximum SON concentration produced during a batch experiment; obtained by subtracting the initially calculated SON (SON_{oc}) from the maximum SON measured (SON_{peak}) during the experiment, mg/l

$dSON_{pkm}$ -- difference between the maximum SON (SON_{peak}) and minimum SON (SON_{min}) measured during batch SON production experiments, mg/l

$dSON_t$ -- SON produced after t hours of aeration; obtained by subtracting the initially calculated SON (SON_{oc}) from the measured SON at aeration time t (SON_t), mg/l

$dSON_t(i)$ -- $dSON_t$ for test condition i, mg/l

$\overline{dSON_t(i)}$ -- average of $dSON_t(i)$ values, mg/l

$dSON_t(i-j)$ -- difference between $dSON_t$ for test condition i ($dSON_t(i)$) and control condition j ($dSON_t(j)$), mg/l

$\overline{dSON_t(i-j)}$ -- average of $dSON_t(i-j)$ values, mg/l

e -- standard error of estimate for linear regression analysis, ± mg/l

f -- decimal fraction of biodegradable influent SON (SON_b) that is not removed by activated-sludge treatment

k_n -- first-order decay rate for SON, day^{-1}

k_s -- first-order decay rate for SCOD, day^{-1}

MLSS -- mixed liquor suspended solids concentration, mg/l

$MLSS_{om}$ -- MLSS measured at time 0 of batch experiment, mg/l

MLVSS -- mixed liquor volatile suspended solids concentration, mg/l

μ -- organism growth rate, day^{-1}

NH_3-N -- ammonia nitrogen concentration, mg/l

NH_3-N_{oc} -- initially calculated NH_3-N, mg/l

NO_2^--N -- nitrite-nitrogen concentration, mg/l

NO_3^--N -- nitrate-nitrogen concentration, mg/l

r -- correlation coefficient

$SBOD_5$ -- soluble five-day biochemical oxygen demand, mg/l

SCOD -- soluble chemical oxygen demand, mg/l

$SCOD_{eq}$ -- SCOD concentration in "equilibrium" with activated-sludge organisms, mg/l

$SCOD_{oc}$ -- initially calculated SCOD, mg/l

$SCOD_q$ -- SCOD initially released by organisms upon dilution with tap water to reach the "equilibrium" SCOD concentration, mg/l

SOC -- soluble organic carbon concentration, mg/l

SON -- soluble organic nitrogen concentration, mg/l

SON_{b} -- biodegradable SON in untreated wastewater, mg/l

SON_{d} -- SON produced during activated-sludge treatment by organism decay, mg/l

SON_{db} -- biodegradable SON_{d}, mg/l

SON_{dr} -- refractory SON_{d}, mg/l

SON_{e} -- SON in effluents from activated-sludge treatment, mg/l

SON_{eq} -- SON concentration in "equilibrium" with activated-sludge organisms, mg/l

SON_{f} -- SON of defined-substrate feed solutions, mg/l

SON_{g} -- SON produced during activated-sludge treatment as a result of substrate oxidation, mg/l

SON_{gb} -- biodegradable SON_{g}, mg/l

SON_{gr} -- refractory SON_{g}, mg/l

SON_{i} -- SON of untreated (influent) wastewater, mg/l

SON_{min} -- minimum SON measured during a batch experiment, mg/l

SON_{ml} -- SON of the activated-sludge mixed liquor used in batch experiments, mg/l

SON_{o} -- SON measured on day 0 of low seed biodegradation study, mg/l

SON_{oc} -- initially calculated SON, mg/l

$\mathrm{SON}_{oc}(i)$ -- SON_{oc} for test condition i

SON_{om} -- SON measured at time 0 of batch experiment, mg/l

SON_{p} -- SON produced during activated-sludge treatment, mg/l

SON_{pb} -- biodegradable SON_{p}, mg/l

SON_{peak} -- maximum SON measured during production batch experiments, mg/l

SON_{pr} -- refractory SON_{p}, mg/l

SON_{q} -- SON initially released by organisms upon dilution with tap water to reach the "equilibrium" SON concentration, mg/l

SON_{r} -- refractory SON in untreated wastewater, mg/l

SON_{rf} -- estimate of refractory SON; used in first-order decay model, mg/l

SON_{t} -- SON measured after t days of low seed biodegradation, mg/l

$\mathrm{SON}_{t}(i)$ -- SON measured after t hours of batch activated-sludge aeration for test condition i, mg/l

SON_{tap} -- SON of tap water, mg/l

SON_{tm} -- SON measured after t hours of batch activated-sludge aeration, mg/l

t -- batch activated-sludge aeration time, hrs, or low seed biodegradation incubation time, days

TBOD_{5} -- unfiltered five-day biochemical oxygen demand, mg/l

TCOD -- unfiltered chemical oxygen demand, mg/l

θ -- hydraulic detention time, hours

θ_{c} -- solids retention time, days

TON -- unfiltered organic nitrogen, mg/l

V_{f} -- volume of feed solution added to batch activated-sludge reactor, liters

V_{ml} -- volume of activated-sludge mixed liquor added to batch activated-sludge reactor, liters

ACKNOWLEDGMENTS

The cooperation of the personnel at the Palo Alto Regional Water Quality Control Plant, the San Jose/Santa Clara Water Pollution Control Plant, the Union District Plant #3, and the South Tahoe Public Utility District Water Reclamation Plant in the collection of samples and use of their facilities for portions of this study is gratefully acknowledged.

SECTION 1

INTRODUCTION

BACKGROUND

In 1972 The United States Congress passed the Federal Water Pollution Control Act Amendments [1] declaring that "it is the national goal that the discharge of pollutants into the navigable waters be eliminated by 1985 [and] it is the national policy that a major research and demonstration effort be made to develop technology necessary to eliminate the discharge of pollutants." As an interim step in fulfilling this national goal, secondary treatment of all municipal wastewaters will be required by 1977. Currently a major effort is being made to identify the pollutants present in secondary effluent and to develop processes for their removal.

There are a large number of organic and inorganic constituents present in secondary effluent which can be discharged into receiving waters. Of these constituents, those containing nitrogen have received a great deal of attention due to their ability to stimulate undesirable algal blooms. The inorganic forms of nitrogen have also been found to act as pollutants in other ways: nitrate can cause methemoglobinemia in infants; nitrite can combine with organic compounds to form nitrosamines, which are potent carcinogens; and ammonia is toxic to fish and can cause lowering of the dissolved oxygen level in receiving waters [2]. These inorganic forms of nitrogen have been extensively studied, and many processes have been developed for their removal. Much less is known regarding the occurrence and removal of the organic forms of nitrogen.

The U.S. Environmental Protection Agency has been given the responsibility of setting standards for the discharge of chemical substances into the environment. In order to set such standards intelligently a great deal of information will be required, to insure both the protection of the public interest and the attainability of the standards. In the case of organic nitrogen, it is desirable to know (1) its concentration and variation in concentration in municipal secondary effluents; (2) if the material is biodegradable; (3) if it can stimulate the growth of algae; (4) if it poses a public health problem; and (5) the degree to which it can be removed by advanced waste-treatment processes.

In a strict literal sense "organic nitrogen" includes any nitrogen atom that is part of an organic molecule. However, the term "organic nitrogen" has been used extensively in the literature to designate organically bound nitrogen in the tri-negative state as measured by the Kjeldahl organic nitrogen analysis [3]. This definition will be adhered to in this study.

There is little information available concerning the concentration and nature of the organic nitrogen present in municipal secondary effluent, although it may constitute more than half of the total nitrogen in a denitrified effluent [4]. A certain portion of this organic nitrogen is in the form of particulate matter which has failed to settle during secondary sedimentation. This fraction of the organic nitrogen is quite easily removed by filtration.

The remainder of the organic nitrogen, which cannot be removed by filtration, is designated as soluble organic nitrogen (SON), and is the subject of this study. SON will be arbitrarily defined as that portion of Kjeldahl nitrogen capable of passing through a 0.45 micron membrane filter. This is an operational definition, and it is quite possible that a significant part of the SON defined in this way is not in true solution, but rather in a colloidal state.

OBJECTIVES

Soluble organic material containing nitrogen represents a significant portion of the nitrogen present in treated municipal wastewaters, but little is known of the effects this material may have on receiving waters, its characteristics, its sources, or methods for its removal. Proposed standards for total nitrogen limitation in treated effluents often include this material, but data relating to its concentration and variation in concentration and studies on methods for its removal are lacking. Thus, information about soluble organic nitrogen is needed in order to establish sound criteria and to design treatment systems for its removal. The objectives of this study were:

1. To determine the concentration and variation in concentration of SON in municipal secondary effluents.
2. To evaluate the potential of biological, physical, and chemical treatment processes and combinations of processes for removal of SON, including activated-sludge treatment, chemical coagulation, chemical oxidation, and adsorption.
3. To determine what portion of SON in secondary effluents is residual from the untreated wastewater and what portion is formed biologically during treatment.
4. To characterize SON chemically and operationally in order to better understand its potential sources, nature, potential ecological effects, and susceptibility to removal by treatment processes.
5. To determine differences and similarities in behavior between SON and the other soluble organic materials present in secondary effluents.

SECTION 2

CONCLUSIONS

SON IN SECONDARY MUNICIPAL EFFLUENTS

1. The concentration of SON in the secondary effluents ranged from 1.1 to 2.1 mg/l, with an average of 1.5 mg/l at four activated-sludge treatment plants, ranging in capacity from 3 to 141 mgd and varying in influent waste characteristics.
2. Diurnal variations in the concentration of SON in the secondary effluents of the three activated-sludge treatment plants evaluated were small, with maximum to minimum ratios ranging from [illegible] to 1.3, and maximum to average ratios ranging from 1.1 to 1.3 mg/l. Variations in SON and SCOD (soluble COD) were very similar in all cases.

SECONDARY EFFLUENT SON REMOVAL BY PHYSICAL AND CHEMICAL PROCESSES

1. A sequence of advanced wastewater-treatment processes designed for wastewater reclamation removed a large amount (perhaps 70-90 percent) of the SON and SCOD from secondary effluents, the bulk of the removal occurred during coagulation and activated-carbon adsorption.
2. A fractionation scheme was developed which separates the SON and SCOD into fractions based on their removal by the various individual processes and combinations of processes.
3. Ferric chloride, lime, and alum removed 42 ± 5, 34 ± 5, and 27 ± 3 percent of SON in secondary effluent, and 32 ± 6, 24 ± 8, and 28 ± 10 percent of the SCOD, respectively. The differences in SON removal between the three coagulants were statistically significant, but differences in SCOD removal were not. Ferric chloride and lime removed significantly more SON than SCOD.
4. Polyelectrolytes produced very rapidly settling flocs, but were of no benefit in removing SON.
5. Bentonite improved SON removal by about 10 percent at a dose of 200 mg/l, but no increased removal of SON was found with kaolinite.
6. Direct addition of ferric chloride to activated sludge resulted in about 30 percent removal of SON at a dose of 200 mg/l, although a

significantly higher removal of SON could be attained by separate coagulation of the supernatant liquor. Direct addition merits consideration for economic reasons.

7. Activated carbon achieved an average SON removal of 71 ± 12 percent, and an average SCOD removal of 81 ± 8 percent, reflecting a significantly higher removal of SCOD than of SON.

8. Cation exchange at neutral pH removed 11 ± 1 percent of the SON and 4 ± 5 percent of the SCOD. Anion exchange at neutral pH removed 12 ± 4 percent of the SON and 30 ± 4 percent of the SCOD.

9. Sequential contacting with ion-exchange resins revealed that the fractions of SON and SCOD removed by the cationic resin at pH 2 and by the anionic resin at pH 12 were significantly different, as were the fractions of SON and SCOD removed by both resins at neutral pH. However, there appeared to be some overlapping of the fractions removed by the two resins at both low and high pH.

10. With a cation-exchange resin, decreasing pH sharply increased the removal of both SON and SCOD, the percentage removed being significantly greater for SON than for SCOD. At pH 2.0, the cationic resin removed 42 ± 7 percent of the SON and 22 ± 4 percent of the SCOD.

11. At neutral pH, cation-exchangeable SON was not adsorbed by activated carbon, anion-exchangeable SON was partially adsorbed by activated carbon, and anion-exchangeable SCOD was almost completely adsorbed by activated carbon.

12. The SON and SCOD remaining after activated-carbon adsorption are not readily attacked by ozone.

13. The fraction of SON removed by cation exchange at neutral pH is also removed by ferric chloride coagulation at pH 10, but not at pH 6. The fractions of SON and SCOD removed by anion exchange are not removed by ferric chloride coagulation at either pH 6 or 10.

14. At pH 7.0, chlorine dosages less than 100 mg/l (below the breakpoint) produced no significant removal of SON and very little (6 ± 2 percent) removal of SCOD. A chlorine to ammonia nitrogen ratio of approximately 20:1 removed an average of 42 ± 4 percent of the SON and 18 ± 7 percent of the SCOD. A slightly lower amount of oxidation of SON was found for a 10.4:1 ratio, but SCOD removal was unaffected by an increase above a ratio of about 10.

15. Potassium permanganate and hydrogen peroxide removed a maximum of 28 ± 3 and 19 ± 3 percent of the SON, respectively, at pH 10.0 and 11.0, respectively. They produced no significant removal of SCOD.

16. Ozonation removed an average of 14 ± 7 percent of the SON and 46 ± 4 percent of the SCOD, at absorbed dosages mostly in excess of 100 mg/L

17. The fractions of SON removable by ozone and chlorine were found to be mutually exclusive. Removal of SON by chlorine was increased 30 percent by preozonation. Ozone removed a considerably greater amount of SCOD than chlorine.

18. Ozonation increased the biodegradability of remaining SON and SCOD by 29 ± 4 and 17 ± 4 percent, respectively.

SON PRODUCTION AND REMOVAL BY BIOLOGICAL TREATMENT

1. From 60 to 70 percent of the SON and 70 to 80 percent of the SCOD in untreated municipal wastewater was removed by activated-sludge treatment; SCOD was in general removed at a faster rate than SON.

2. A minimum in the concentrations of effluent SON is predicted to result when municipal activated-sludge plants are operated at conventional loadings, such as aeration times of six hours and solids retention times of four to ten days. Higher or lower loadings should result in increased SON concentrations.

3. Under the above optimal conditions and with normal operation, about two-thirds of the effluent SON represents residual organics from the influent wastewater and the other one-third represents materials produced biologically during activated-sludge treatment. The effluent SON is relatively refractory to biological degradation.

4. During activated-sludge plant start-up and following plant upsets, effluent SON concentrations can increase by several mg/l as a result of biological production. The incremental SON produced during these periods is fairly biodegradable.

5. Under normal operating conditions at conventional loadings, about one-half of the SON produced biologically during treatment results from organism decay and the other one-half represents biological exudates which approach an "equilibrium" concentration with the living cells. The equilibrium concentration is independent of cell concentration but is dependent upon culture characteristics.

6. Some evidence suggests that a separate SON fraction may be produced biologically as a response to substrate oxidation. However, this fraction is generally small in comparison with the fractions produced by decay and in equilibrium with the cells.

7. The quantity of SON released during organism decay is a function of mixed liquor volatile solids concentration and time of aeration. This material is refractory to biological oxidation.

8. The extent of biological production of SON is consistent for a given activated-sludge culture, but varies greatly between cultures. Thus, generalizations developed with one culture may not apply to other cultures, even if treating a similar wastewater.

CHARACTERISTICS OF EFFLUENT SON

1. Less than ten percent of effluent SON was comprised of free or combined amino acids.

2. Evidence suggests that 15 to 30 percent of the SON was represented by nucleic acid degradation products, specifically nucleic acid bases and other heterocyclic nitrogen-containing compounds.

3. Effluent SON was much more refractory (40 to 50 percent with first-order decay rates for the remainder of 0.014 day^{-1}) than SON in untreated wastewater (18 to 38 percent with decay rates for the remainder of 0.08 to 0.16 day^{-1}).

4. Fifty to sixty percent of the SON and SCOD in treated and untreated municipal wastewater had apparent molecular weights less than 1800 as measured by Sephadex gel filtration.

5. About 25 percent of the SON had an apparent molecular weight between 165 and 340 and a high ratio of SON to SCOD of 0.31 ± 0.11, compared with the typical ratio for other ranges of 0.045 ± 0.015. This and other evidence suggests this material consists largely of heterocyclic nitrogen-containing compounds such as nucleic acid degradation products.

6. The increased concentration of SON and SCOD produced biologically during activated-sludge culture start-up had a generally higher molecular weight distribution than normal secondary effluent, with considerably more organics in the greater-than-1800 molecular weight range.

7. The organic materials associated with effluent SON and SCOD were characterized operationally by various physical and chemical processes. About 70 percent of the SON and 80 percent of the SCOD represented relatively non-polar materials (aside from charge) removable by activated-carbon adsorption, of which about one-third had a predominantly negative charge and the remainder a neutral charge. The remaining 30 percent of the SON and 20 percent of the SCOD was relatively polar, one-third being characterized as positive and the remainder as neutral.

SECTION 3

RECOMMENDATIONS

1. Judgement should be exercised in the formulation of effluent standards which may specify or otherwise include limitations on the discharge of SON to receiving waters. The SON contained in effluents from secondary treatment of municipal wastewaters is generally low in concentration and also is quite refractory or slow to degrade biologically. No significant environmental problems have yet been shown to result from discharge of this material to receiving waters. While up to 80 percent of this material may be removed by physical and/or chemical treatment, the construction and operational costs for the required facilities would be high.

2. A relatively large fraction of the SON-containing compounds in secondary effluents appears to be degradation products of nucleic acids. Further research to determine the nature of this particular material and the effect chlorination may have on organisms which may re-incorporate this material into their cellular components appears worthy.

3. A large fraction of SON in secondary effluents appears to be formed during biological treatment of wastes. Perhaps a significant portion of the SON arriving at treatment plants is also produced during biological decomposition of human waste products during transport to the treatment plant. More detailed characterization of such biologically produced organics is recommended in order to obtain a better understanding of the naturally produced refractory organic materials occurring in natural waters and treatment plant effluents.

SECTION 4

SOURCES, OCCURRENCE, AND CHEMICAL NATURE OF ORGANIC NITROGEN

INTRODUCTION

This section reviews the sources and concentration of organic nitrogen in raw wastewater and secondary effluents. Since much of the organic nitrogen is of natural origin, the characteristics of organic nitrogen found in soils, sediments, surface waters, and marine environments is briefly examined. This is followed by a discussion of the chemical nature of organic nitrogen.

SOURCES OF ORGANIC NITROGEN

Urine and feces are the two major sources of organic nitrogen in municipal wastewater. Painter et al. [14] estimated the contribution of organic nitrogen to be 1-2 g/day/adult from feces and 0.5-3 g/day/adult from urine. These and other sources of organic nitrogen are listed in Table 1, together with the names of compounds or classes of compounds thought to be present.

Sources of organic nitrogen other than urine and feces may also be important. Numerous commercially available chemicals are frequently poured down household drains and sewers. Urban runoff has been found to have an organic nitrogen concentration of 1-9 mg/l [2], and may be important in treatment plants with a combined sewer system or to receiving waters where runoff is not treated. The extra-cellular material of organisms and their cell fragments also contribute to the organic nitrogen in wastewater.

ORGANIC NITROGEN IN RAW WASTEWATER

Painter et al. [14], Hunter and Heukelekian [15], Rickert and Hunter [16], and Helfgott et al. [13] used various techniques to separate domestic sewage into a number of different fractions. The percentages of the total organics which they found in the soluble fraction are shown in Table 2. Hunter and Heukelekian [15] found a smaller percentage of the organic nitrogen in the soluble fraction than other investigators, but this may have been due to environmental factors such as temperature or travel time in the sewer, or to differences in the separation techniques.

The class of compounds which has received perhaps the widest attention in raw wastewater is the amino acids [5,6,13,14,15,17,18]. Kahn and Wayman [18] found 13 amino acids in raw wastewater in concentrations of 10-15 mg/l, but

TABLE 1. SOURCES OF ORGANIC NITROGEN IN MUNICIPAL WASTEWATER

Source	Nitrogenous Compounds Present	References
Urine	Urea, uric acid, creatine, amino acids	5,6
Feces	Amino acids, biotin, folic acid, nicotine acid, pantothenic acid, riboflavin, thiamine, purine bases	5
Industrial Process Effluents	Aliphatic amines, arylamines, heterocyclic amines, isocyanates, amino acids, urea, thiourea	2
Urban Runoff		2
animal excreta	(See feces)	
plant matter		
fertilizers	Urea	2
chemicals	Cationic detergents (amino, quaternary ammonium compounds)	2
Bacteria, Algae, Fungi		
exudates	Hydroxyamino acids, proteins, amino acids, amides	7,8,9,10
cell walls	Muramic acid, amino sugars	6,11,12,13,14
cell fragments	Nucleic acids, chitin	6

only 7 of these remained after primary treatment in concentrations of about 5 mg/l, indicating that the amino acids were largely present as particulate matter. They also found approximately 41 percent of the amino acids in the free form, although other investigators [6,17,18] have found only a few percent of the proteinaceous matter in raw sewage to consist of free amino acids. This discrepancy is attributed to environmental factors [18].

Hanson and Lee [6] found averages of 43% and 54% of the organic nitrogen in the raw wastewater at two primarily domestic treatment plants in the form of urea and alpha amino acids. The ranges of concentrations for various nitrogen forms in the two raw wastewaters are presented in Table 3.

In addition to amino acids, many other nitrogenous compounds have been identified in raw wastewater. Hunter [5] has listed a number of these as shown in Table 4.

ORGANIC NITROGEN IN SECONDARY EFFLUENT

It is anticipated that the organics present in secondary effluent differ greatly from those found in raw wastewater. The differences are manifested in the magnitude of the gross parameters used to measure the organics, in the relative size of the soluble fraction, in the percentage of organics classified in any of the various schemes, and in the number and concentration of specific chemical compounds present.

TABLE 2. PERCENTAGE OF TOTAL ORGANICS IN THE SOLUBLE FRACTION OF RAW WASTEWATER

Organic Nitrogen		Organic Carbon		Chemical Oxygen Demand		Volatile Solids		Reference
Total mg/1	Percent Soluble	Total mg/1	Percent Soluble	Total mg/1	Percent Soluble	Total mg/1	Percent Soluble	
35	39	412	29	-	-	-	-	Painter et al. [14]
27	37	311	29	-	-	-	-	Painter et al. [14]
-	-	-	42	-	40	116	48	Rickert and Hunter [16]
10.9	53	-	-	299	43	232	48	Helfgott et al. [13]
-	24	-	-	-	25	-	37	Hunter and Heukelekian [15]
-	20	-	-	-	23	-	42	Hunter and Heukelekian [15]
-	43	-	-	-	-	-	-	Hanson and Lee [6]

TABLE 3. NITROGEN FORMS IN TWO RAW WASTEWATERS [6]

Plant:	Nine Springs			Cross Plains		
Population Services:	200,000			950		
Number of Samples:	13			8		
	Avg.	Min.	Max.	Avg.	Min.	Max.
NH_3-N, mg/l	11.2	6.6	14.4	25.9	16.8	30.0
Urea-N, mg/l	1.4	0.0	4.9	6.1	3.9	10.3
Org-N, mg/l	7.3	5.1	18.7	16.1	10.9	21.8
α-amino acid-N, mg/l	2.3	0.8	7.4	3.0	2.4	4.3
TKN, mg/l	18.4	11.7	31.2	42.0	27.7	49.9

TABLE 4. NON-AMINO ACID NITROGENOUS CONSTITUENTS OF DOMESTIC WASTEWATER [5]

Compound	Concentration, mg/l
Urea	2-16
Muramic acid	0.5
Amino sugars	1.2-2.2
Uric acid	0.2-1.0
Hippuric acid	present
Xanthine	trace
Indole	0.00025
Skatole	0.00025
Aliphatic amines	0.1
Creatine-creatinine	0.2-7.0
Organic bases	3.4
Thiamine	0.029
Riboflavin	0.022-0.044
Niacin	0.135
Cobalamin	0.0008
Biotin	0.0003
Pantothenic acid	present
Folic acid	present

A typical activated-sludge plant can be expected to achieve approximately 85-95% removal of five-day biochemical oxygen demand (BOD_5) [24], and also significant percentages of organic nitrogen, organic carbon, chemical oxygen demand (COD), and volatile solids. The concentrations of some of these parameters found by various investigators are shown in Table 5.

The information available regarding the concentration of organic nitrogen in secondary effluent is quite sparse. Several investigators have measured organic nitrogen in nitrified samples, but nitrate interferes in the organic nitrogen analysis (see Appendix B). Bunch et al. [23] do not state whether or not the plants they sampled were nitrifying. The effluent studied by Rebhun and Manka [22] originated from a raw wastewater with a COD of 1200 mg/l and was treated with a trickling filter, thus producing the high concentrations of organics shown in Table 5. The organic nitrogen concentration of 6.1 mg/l reported by Beckman et al. [19] is attributed to very cold temperatures and failure of the organisms to hydrolyze protein.

A comparison of Table 5 with Table 2 indicates that a much larger percentage of the organics in secondary effluent are soluble. Soluble generally refers to materials passing a 0.45 μm filter. It is not known how much of the soluble organic matter present in the effluent was originally present in the raw wastewater and how much of it was produced by bacteria during the treatment process. A number of investigators have shown that microorganisms are capable of excreting extracellular organic matter [7,8,9,10] and some of the organics in secondary effluent may be comprised of these exudates, as well as cell walls and fragments of microorganisms [12,13].

Many soluble organic compounds identified in raw wastewater are not found in treated effluents. Urea is rapidly hydrolized by the enzyme urease, in some cases before it reaches the treatment plant [6], and does not appear in treated effluent. Free amino acids identified in raw wastewater are not found in secondary effluents [17,18]. Rudolphs and Chamberlin [25] found the nitrogenous degradation products indole and skatole in raw wastewater, but detected only trace amounts of them in treated effluents.

Secondary effluent organics have been characterized into known classes of compounds as shown in Table 6. Only 35-50% of the soluble organics could be classified in this way, the unclassified organics being generally labelled as humic substances. Bunch et al. [23] concluded that 40% of the soluble COD and 50-70% of the organic nitrogen comprised high molecular weight compounds since they would not dialyze within four days.

For the three biological processes studied (trickling filter, stabilization pond, and extended-aeration activated sludge), Manka et al. [26] showed that the distribution of the main soluble organic fractions (in secondary effluent) is similar for the various biological treatment units. Manka et al. [26] and Rebhun and Manka [22] classified 19-25% of the soluble organics as proteins; however, this estimate may be high since fulvic acid has been found to interfere in the Lowry [27] test which they used to measure proteins [28].

TABLE 5. TOTAL AND SOLUBLE ORGANICS IN SECONDARY EFFLUENT

Organic Nitrogen		Organic Carbon		Chemical Oxygen Demand		Volatile Solids		Reference
Total mg/1	Percent Soluble	Total mg/1	Percent Soluble	Total mg/1	Percent Soluble	Total mg/1	Percent Soluble	
3.1†	32†	26.9	52	-	-	-	-	Painter et al. [14]*
-	-	-	69	-	74	62	67	Rickert and Hunter [16]
4.2†	26†	-	-	81.8	78	98.3	87	Helfgott et al. [13]
6.1	-	-	-	-	-	-	-	Beckman et al. [19]
2.6†	-	-	-	29.9	82	-	-	Adrian and Hodges [20]
3.8†	-	-	-	45	-	-	-	Esmond and Wolf [21]
18.0 (soluble)	-	-	-	185 (soluble)	-	-	-	Rebhun and Manka [22]*
1.2 (soluble)		-	-	48.9 (soluble)		-	-	Bunch et al. [23]**

*Trickling filter effluent (others are activated-sludge effluents).

†Nitrified effluents; nitrates interfere in the organic nitrogen analysis.

**Average of 7 trickling-filter and activated-sludge samples (range of organic nitrogen was 0.34-1.82).

TABLE 6. CHARACTERIZATION OF SOLUBLE SECONDARY EFFLUENT ORGANICS

Reference:	Bunch et al. [23]	Rebhun and Manka [22]	Manka et al. [26]
Number of Samples:	7	3	11
Secondary Treatment:	Trickling filter or activated sludge	High-rate trickling filter	High-rate trickling filter, stabilization pond, extended-aeration, activated sludge
Sample Treatment:	Whatman #5 filter, vacuum concentrate 20X	Centrifuge, vacuum concentration 20X, 0.45 μ millipore	Centrifuge, vacuum concentration 20X, 0.45 μ millipore
Soluble COD, mg/l	24-86	185 (typical)	105-167
Ether extractables	< 10%	8%	10-20%
Proteins	< 10%	22%	19-25%
Carbohydrates	< 5%	12%	4-8%
Tannins and Lignins	< 5%	2%	1-2%
ABS	~ 10%	14%	11-21%
Humic substances	65%	40-55%	31-51%

All concentrations reported as equivalent COD.

ORGANIC NITROGEN IN SOILS, SEDIMENTS, SURFACE WATERS, AND THE MARINE ENVIRONMENT

The effluent from wastewater treatment plants is usually discharged into a stream, lake, or ocean, and organic nitrogen is thus released into the environment, where it can accumulate in sediments, enter water supplies, or eventually reach the ocean. Some of the organic nitrogen in soils may be similar to that in secondary effluents, due to the intense activity of microorganisms in the soil. While there are undoubtedly differences in the origin of the organic nitrogen from these various sources, it is quite possible that similarities exist in the final refractory compounds and that they share a common ultimate fate.

Siegel and Degens [33] found dissolved amino compounds in sea water in a combined state, and postulated that a sizable portion existed in complexes of the phenol-quinone type.

Minear [34] concluded that a sizable percentage of the high-molecular weight material which he isolated from lake water appeared to be deoxyribonucleic acid or its fragments.

Bremner [35] and Keeney [11] reported that about 98% of the nitrogen in soils and sediments is present as organic nitrogen. Keeney [11] studied the distribution of amino acids and hexosamines in lake sediments. Bremner [35, 36] found that a minimum of one-third of the organic nitrogen in soils was in the form of "protein-like combinations," and about 3-10% was present as amino sugars. Bremner also found that 20-60% of soil organic nitrogen was not dissolved by acid hydrolysis (possibly part of this fraction was composed of heterocyclic nitrogen), but the major fraction of the dissolved organic nitrogen was comprised of amino acids [36]. Other compounds found in soils include amines, purine bases, pyridine and pyrimidine derivatives, and heterocyclic compounds [35].

Stevenson and Goh [37], Otsuki and Hanya [38], and Ishiwatari [39] have found indications of the presence of proteins in humic substances from a number of lake sediments and soils.

CHEMICAL NATURE OF ORGANIC NITROGEN IN SECONDARY EFFLUENTS

A large portion of the organic nitrogen in secondary effluent remains unclassified. Some organic nitrogen has been classified as protein by means of the Lowry test [22,26,27], by acid hydrolysis to yield amino acids, or by multiplying non-dialyzable Kjeldahl organic nitrogen by a factor of 6.25 [23]. None of these methods provides conclusive evidence that the material is actually protein. Furthermore, a large fraction of the organic nitrogen does not seem to be present in the form of combined amino acids, although some of this nitrogen may be present in heterocyclic compounds. It is quite conceivable that a number of chemical reactions or complex formations may have altered the nature of the organic nitrogen considerably from what it was in the raw wastewater. A number of investigators have postulated or observed chemical or physical phenomena which are of interest.

The Browning (or Maillard) reaction between amino acids and carbohydrates to form brown unsaturated polymers and co-polymers of nitrogen has been studied extensively by Hodge [40] and discussed by Bremner [35], Hanson and Lee [6], and Stevenson and Tilo [41] in their studies of organic nitrogen in soils, domestic wastewater, and sediments, respectively.

Dugan [42] studied the extracellular polymers produced by floc forming bacteria, and concluded that they were water soluble polysaccharides. The polymers were shown to be capable of adsorbing dissolved organics, such as amino acids. Dean [12] found substantial quantities of non-protein nitrogen present in the amino sugars of cell walls and bacterial slime. Baier [43] suggested that amino acids, amines, proteins, and polypeptides may be effective as coupling agents, promoting adhesion by chemical or physical processes in aqueous solution.

Putnam and Neurath [44], using electrophoretic techniques, observed complex formation between proteins and detergents on both the acid and the alkaline side of the isoelectric points of the proteins.

Secondary treatment can conceivably produce a myriad of chemical reactions, many of them biologically mediated, in the presence of numerous ions, ligands, surfactants, and functional groups. The result is the production of amorphous humic substances which defy classification by traditional methods.

SECTION 5

MATERIALS AND METHODS

PHASES OF THE STUDY

This study on soluble organic nitrogen characteristics and removal was conducted in four phases. The first was concerned with the variation in concentration of SON in secondary effluents from municipal wastewater treatment plants. The second was an evaluation of various physical and chemical processes, individually and in combination, for the removal of SON. The third phase evaluated the biodegradability of SON-containing organics in primary and secondary municipal wastewater effluents, and the extent of production of SON during biological treatment. The last phase represented an attempt to characterize the chemical characteristics of soluble organic nitrogen. Different experimental methods were used for each phase and are described under each phase of the study. However, many of the analytical procedures used were similar for the different phases and are described below.

ANALYTICAL METHODS

Standard Methods

The following analyses were carried out as described in Standard Methods [3]: organic nitrogen (except as noted later); chemical oxygen demand (COD), using one-tenth strength potassium dichromate and ferrous ammonium sulfate; total organic carbon (TOC), using a Beckman organic carbon analyzer; five-day biochemical oxygen demand (BOD_5); ammonia (distillation with Nesslerization); nitrate (Cadmium Reduction method) and nitrite (except as noted later); pH, using a Beckman Model 1009 Electromate pH meter equipped with a Beckman low sodium-error electrode; alkalinity, by titration to pH 4.0; total suspended solids (TSS); volatile suspended solids (VSS); mixed-liquor suspended solids (MLSS); mixed-liquor volatile suspended solids (MLVSS); residual chlorine (Iodometric method); methylene-blue active substances (MBAS); hydroxylated aromatics (Tannin and Lignin), using Hach TanniVer III reagent in place of tannin-lignin reagent; and turbidity, using a Hach Model 2100A Turbidimeter which gave a reading of 0.2 nephelometric turbidity units (NTU) with deionized water. Residual ozone was measured using the iodometric method for residual chlorine.

SON, soluble COD (SCOD), soluble BOD_5, and soluble TOC were measured using the same procedures as for whole samples. Analysis for these soluble constituents was performed on samples which had been filtered through a 0.45 micron membrane filter, either Millipore HAWP or Pall DFA 3001 AXA. These two filters were found to produce roughly equivalent values of SON and SCOD on samples of primary and secondary effluent. Most filtered samples were first passed through a glass fiber filter (Reeve Angel 934AH) to prevent rapid clogging of the membrane filters.

The water used for all reagents, blanks, dilutions, and rinsing of glassware was deionized tap water. All chemicals used equaled or exceeded the grades specified by Standard Methods [3]. All glassware was cleaned with a dichromate-sulfuric acid cleaning solution. Colorimetric analyses were performed with a Bausch and Lomb Spectronic 70 Spectrophotometer.

Nitrate was found to interfere in the SON analysis at concentrations above 10 mg/l. Thus, nitrified samples could not be accurately analyzed for their SON content. Nitrite was found not to interfere in the analysis.

The precision of the SON and SCOD analyses is of great importance in evaluating the experimental data, since these were the two most important constituents investigated in the majority of the experiments. The standard deviations of SON and SCOD analyses run on replicate samples are presented in Table 7. Calculation of the combined standard deviation is shown in Appendix A. Also contained in Appendix A are calculations of the confidence levels (CI) of the SON and SCOD analyses, the estimated error for both analyses, and a discussion of significance testing.

To be considered significant a difference between two analyses must exceed the value shown in Table 8. The term "significant" will be applied to differences which have a 95% CI. As discussed in Appendix A, the significance of differences in percent removal is dependent upon initial concentration. Differences which are significant at different confidence levels will be stated where appropriate.

TABLE 7. PRECISION OF SON AND SCOD ANALYSES

Type of Sample	Analysis	Number of Replicates	Average Value mg/l	Standard Deviation mg/l	Combined Standard Deviation mg/l
Secondary Effluent	SON	6	0.95	0.03	0.03
Secondary Effluent	SON	9	1.08	0.03	
Act. Carbon Effl.	SON	4	0.19	0.03	
Act. Carbon Effl.	SON	8	0.37	0.03	
Secondary Effluent	SCOD	8	23.5	0.5	0.4
Act. Carbon Effl.	SCOD	8	4.8	0.3	

TABLE 8. SIGNIFICANT DIFFERENCES IN SON AND SCOD ANALYSES

Analyses	Differences to be exceeded, mg/l	
	95% Confidence	99% Confidence
SON	0.08	0.11
SCOD	0.9	1.3

The recovery of a standard (valine) concentration of SON (1.0 mg/l) in Palo Alto Secondary Municipal Effluent (PASE) was checked and found to be 99%. showing that the constituents of PASE do not appear to interfere in the analysis. An initial step in the SON analysis is removal through distillation of ammonia, which otherwise would create a positive interference. To determine if pH 7.4 was high enough during distillation to ensure complete ammonia removal, a series of samples was distilled at various pH values as shown in Table 9. The pH of the first distillation had no significant effect on the value obtained for PASE samples.

TABLE 9. EFFECT OF INITIAL AMMONIA DISTILLATION pH ON THE OVERALL SON ANALYSIS

Sample	pH of Distillation	SON, mg/l
1	7.4	1.28
2	9.0	1.28
3	10.0	1.26
4	11.0	1.25
5	12.0	1.25

Methods for the Technicon AutoAnalyzer

SON and ammonia values were on occasion determined with the aid of a Technicon AutoAnalyzer (Technicon Instruments Corporation, Terrytown, New York) capable of analyzing a large number of samples semi-automatically. The same instrument was frequently used to determine nitrite and nitrate.

SON was determined by distilling and digesting the samples in a Technicon Block Digester (Technicon Instruments Corp.), and analyzing the ammonia in the digestate. Samples were placed in digestion tubes containing a phosphate buffer (Ammonia Determination: Standard Methods [3]) and heated for approximately 1.5 hours at 160°C to distill off the ammonia. Digestion reagent (Organic Nitrogen Determination: Standard Methods [3]) was then added, and the samples digested for one hour at 370°C. After cooling, the digestate was diluted to a known volume with deionized water, and the ammonia nitrogen concentration was measured as described below. Four replicates of a 1.00 mg/l SON standard gave results of 1.00 ± 0.03 mg/l when measured by this technique. When 20 mg/l of ammonia nitrogen was added to the SON standard, recovery of SON was 1.03 ± 0.10 mg/l, indicating no interference from ammonia nitrogen.

Ammonia nitrogen was determined by measuring the intensity of the "emerald green" color developed by the reaction between ammonia, sodium salicylate, sodium nitroprusside, and sodium hypochlorite at a pH of 12.8-13.0 (Industrial Method No. 375-74W, Technical Instruments Corp.). Five replicates of a 1.00 mg/l ammonia nitrogen standard gave results of 1.00 ± 0.02 mg/l when measured by this method.

Nitrite was determined by reaction with sulfanilamide under acidic conditions to form a diazo compound which was then coupled with N-1-naphtylethylenediamine dihydrochloride to form a reddish-purple azo dye measured colorimetrically (Industrial Method No. 100-70W, Technicon Instruments Corp.).

Nitrate was determined by reducing the nitrate to nitrite by means of a miniature cadmium reduction column packed with a cadmium-mercury amalgam as described in Standard Methods [3]. The nitrite was then measured by the method just described.

Other Methods

Ozone dosage was determined by diffusing the gas stream through 250 ml of 2% potassium iodide in a glass column. The contents were then emptied into a flask, acidified with 2 ml of concentrated sulfuric acid, and the iodine was titrated with 1N sodium thiosulfate, using a starch indicator to determine the endpoint.

Proteins were generally measured using the technique developed by Lowry et al. [46]. Carbohydrates were determined using the anthrone procedure as described by Pfeffer [47] for non-cellulose carbohydrates.

Sample Preservation

Most of the samples in this study were analyzed immediately following the experiment in which they were collected; however, at times it was necessary to store samples for a short period of time. Such samples were refrigerated at 4°C (Standard Methods [3]), and were found to undergo no changes in SON or SCOD concentrations at that temperature. Hunter and Heukelekian [15] found a temperature of 4°C to be effective in preventing losses of organic matter in filtered raw sewage, which is much less stable than secondary effluent. (The only exception to this procedure was that some of the samples collected during the first phase of the study were preserved with mercuric chloride and stored on ice, but this practice was subsequently discontinued for reasons described in Section 6.)

SECTION 6

SON IN MUNICIPAL SECONDARY EFFLUENTS

INTRODUCTION

This section summarizes the characteristics of wastewaters after various stages of treatment for four different municipal wastewater-treatment plants. Raw and primary wastewaters were analyzed to evaluate waste strength and to provide information on the efficiency of secondary treatment in removing SON. The diurnal variation of SON was determined after various stages of treatment to provide data for the design of processes for its removal. The organic and inorganic characteristics of Palo Alto secondary effluent (PASE) were examined in greater detail, since this effluent was used for the laboratory experiments.

DESCRIPTION OF THE SAMPLING SITES

Samples were collected from four activated-sludge plants, each of which differed from the others in size and waste characteristics. These plants are: (1) Palo Alto Regional Water Quality Control Plant; (2) San Jose/Santa Clara Water Pollution Control Plant; (3) Union Sanitary District Plant #3; and (4) South Tahoe Public Utility District Water Reclamation Plant.

The Palo Alto Regional Water Quality Control Plant is a typical complete-mix activated-sludge plant located in Palo Alto next to the San Francisco Bay. It serves the communities of Palo Alto, Mountain View, and Los Altos, and is designed to treat an average dry-weather flow of 1.53 m^3/s (35 mgd). The plant receives a significant amount of industrial waste, and the waste received at the plant is quite "weak" in terms of BOD and COD.

The San Jose/Santa Clara Water Pollution Control Plant is located approximately 9 km north of San Jose's central business area on a site encompassing approximately 40×10^4 m^2. The 8.3×10^8 m^2 service area tributary to the plant includes the cities of San Jose and Santa Clara, and the major portion of the Santa Clara Valley extending southward 40 km from the San Francisco Bay. The plant is designed to treat an average dry-weather flow of 7.0 m^3/s (160 mgd) and at the time the samples were taken was being operated as a conventional activated-sludge plant. (During canning season, the Kraus Process is employed.)

Union Sanitary District Plant #3 is located in Union City, California. The plant is designed for a flow of 0.13 m^3/s (3 mgd) and receives both domestic and industrial waste. The influent wastewater is screened and then pumped into a vacuator, which provides the equivalent of primary treatment by employing both flotation and settling. Next are four aeration tanks which during this study were operated in parallel as in the conventional activated-sludge process. Activated sludge is wasted to the influent wet well, which can cause certain parameters to be quite high in the primary effluent. During normal operation, supernatant liquor from digested-sludge storage lagoons is recycled back to the primary settling tanks. During sampling for this study, however, the supernatant liquor was not being returned.

The South Tahoe Public Utility District Water Reclamation Plant is a 0.33 m^3/s (7.5 mgd) advanced wastewater-treatment plant located just south of Lake Tahoe in California. The raw wastewater, comprised almost entirely of domestic sewage, receives primary and conventional activated-sludge treatment prior to advanced waste treatment consisting of lime clarification, ammonia removal, recarbonation, chlorination, filtration, and activated-carbon adsorption. During sampling for this study, the ammonia stripping process was not in operation.

SAMPLE COLLECTION AND STORAGE PROCEDURES

At each plant except Tahoe, samples were taken of the raw influent wastewater and the primary, secondary, and final (chlorinated) effluents. At the Tahoe plant samples were taken of the raw influent wastewater, secondary effluent, lime-treated effluent, chlorinated effluent, filtered effluent, and activated-carbon effluent. Grab samples were taken from each location every two hours for twenty-four hours. A portion of each sample was filtered through a glass fiber filter (Reeve Angel 934AH) and both filtered and unfiltered portions were preserved on ice. Composite samples were prepared by mixing the grab samples in volumes proportionate to flow, the remaining portions of the grab samples being stored and analyzed individually for measuring diurnal variations in characteristics. After the samples reached the laboratory, the filtered samples were passed through a 0.45 micron filter (Millipore HAWP or Pall DFA 3001 AXA), the filtrate being saved for analysis of the soluble constituents.

Standard Methods [3] recommends that samples stored for nitrogen analyses be cooled to just above freezing, and that acid or mercuric chloride be added to the sample. EPA [48] recommends mercuric chloride plus cooling to 4°C for preservation of nitrogen forms. Howe and Holley [49] found both mercuric chloride and sulfuric acid to be effective in preserving the various forms of nitrogen.

Both acid and mercuric chloride were tested as preservatives for SON in raw sewage, and both were found to be equally acceptable. It was decided not to use acid because (1) it would not permit measurement of pH, alkalinity, or nitrites, and (2) it might cause a significant shift in the relative amount of soluble and particulate matter in the sample.

Prior to April 24, 1975 the samples (except for portions stored separately on ice only for BOD_5 analysis) were preserved with 40 mg/l of mercuric chloride, in addition to being cooled with ice. However, it was noted that the addition of mercuric chloride to a wastewater sample which had been filtered through a glass fiber filter and stored on ice caused the sample to become cloudy; and within a day after it had been added a precipitate became visible. If the sample was then filtered through a 0.45 micron filter, a significant loss of organic matter resulted. For this reason the addition of mercuric chloride was stopped and after April 24, 1975 only ice was used as a preservative. Hunter and Heukelekian [15] studied the preservation of filtrates of raw sewage and found that no losses of organic matter occurred at 4°C.

GENERAL CHARACTERISTICS OF THE INFLUENT AND PRIMARY EFFLUENT WASTEWATERS

The general characteristics of the influent and primary effluent wastewater, as determined from analysis of the 24-hour composite samples taken from each of the four plants, are presented in Table 10. The plants sampled ranged

TABLE 10. GENERAL CHARACTERISTICS OF THE INFLUENT AND PRIMARY EFFLUENT WASTEWATERS†

Parameter	Palo Alto*		San Jose/ Santa Clara*		Union City*		Tahoe
	Influent	Primary Effluent	Influent	Primary Effluent	Influent	Primary Effluent	Influent
SON	5.2	4.4	5.5	4.8	5.2	4.2	2.9
Ammonia-N	28.7	28.4	26.7	26.7	19.9	20.8	23.9
Organic-N	12.2	8.3	20.0	13.4	12.2	18.9	9.3
Nitrate-N	1.8	1.3	0.0	0.2	0.2	0.0	0.2
Nitrite-N	0.38	0.40	0.03	0.08	0.21	0.07	0.02
Total Nitrogen	43.1	38.4	46.7	40.4	32.5	39.8	33.4
COD	381	207	761	451	570	539	388
SCOD	94	86	234	220	128	107	123
Soluble TOC	37	29	84	81	53	46	-
BOD$_5$	171	125	306	240	212	203	186
Soluble BOD$_5$	49	42	148	143	82	68	64
pH, units	7.6	7.7	7.3	7.6	7.6	7.4	
Alkalinity	209	209	284	287	323	328	160
TSS	172	72.5	349	109	205	258	144
VSS	143	60.5	267	85	166	220	138
VSS, percent	82	81	78.5	80	82	85	96
Avg. Flow, m^3/s	1.17		3.7		0.16		0.18

†Values represent mg/l unless otherwise indicated.

*Values are averages of two samples taken on different days.

in flow from 0.16 to 3.7 m^3/s (3.6 to 80 mgd), in raw wastewater SON from 2.9 to 5.5 mg/l, and in waste strength from a COD of 381 to 761 mg/l.

GENERAL CHARACTERISTICS OF SECONDARY EFFLUENT

Secondary effluent is used to designate both activated-sludge effluent and chlorinated effluent. The general characteristics of the secondary effluents, as determined from analysis of the 24-hour composite samples taken from each plant, are presented in Table 11. Although these plants differed in size, raw-wastewater characteristics, loading, temperature, and MLSS, the

TABLE 11. GENERAL CHARACTERISTICS OF THE SECONDARY EFFLUENTS[†]

Parameter	Palo Alto[*]		San Jose/ Santa Clara[*]		Union City[*]		Tahoe
	Activ.-Sludge Effluent	Chlori-nated Effluent	Activ.-Sludge Effluent	Chlori-nated Effluent	Activ.-Sludge Effluent	Chlori-nated Effluent	Activ.-Sludge Effluent
SON	1.65	1.78	1.64	1.79	1.55	1.51	1.17
Ammonia-N	25.7	25.0	21.4	21.8	19.2	18.5	21.4
Organic-N	3.3	3.2	3.4	3.3	4.2	4.6	4.2
Nitrate-N	0.2	0.2	1.5	1.7	0.0	0.1	0.1
Nitrite-N	0.07	0.03	1.40	0.45	0.01	0.01	0.31
Total Nitrogen	29.3	28.4	27.7	27.2	23.4	23.2	26.0
COD	53	52	59	57	111	118	84
SCOD	27	30	36	39	44	41	25
Soluble TOC	9	10	15	17	15	16	10
BOD_5	11	-	9	-	20	-	41
Soluble BOD_5	3	-	2.8	-	5	-	2
pH, units	7.4	7.4	7.8	7.7	7.5	7.5	7.5
Alkalinity	211	200	268	261	317	300	166
TSS	22	19	19	16	34	36	56
VSS	20	17	17	15	27	28	48
VSS, percent	88	89	92	93	82	80	86
Activated-Sludge System Characteristics:							
Avg. Flow, m^3/s	1.17		3.7		0.16		0.18
Temperature, °C	19.5		22.8		20.5		16.5
MLSS	1510		3350		3700		2170
MLVSS	1188 (78%)		2415 (72%)		2930 (79%)		1840 (85%)
Loading, kg BOD_5/kg MLVSS/day	0.38		0.51		0.37		0.45

[†] Values represent mg/l unless otherwise indicated.

[*] Values are averages of two 24-hour composite samples taken on different days.

general characteristics of the effluents were similar, with the major differences apparently being due to the relative efficiencies of the secondary clarifiers. The chlorinated effluents did not differ significantly from the non-chlorinated effluents.

CONCENTRATION OF SON

The concentration of SON found in each of the filtered composite samples is shown in Table 12. The raw wastewaters ranged from 2.9 to 6.3 mg/l SON with an average concentration of 4.9 mg/l. The SON of the primary effluents averaged 4.5 mg/l, reflecting an average 15% removal. This removal may have been due to biological oxidation, adsorption onto solids, or a combination of both. The available data provide no information regarding these mechanisms.

The concentration of SON in secondary effluent averaged 1.5 mg/l, ranging from 1.2 to 2.1 mg/l and reflecting a 69% decrease through the overall treatment process. The concentration of SON in the chlorinated effluents was not significantly different from that of the non-chlorinated effluents.

The concentration of SON in the secondary effluent remained within the rather narrow range of 1-2 mg/l despite differences in raw wastewater strength,

TABLE 12. SUMMARY OF SON CONCENTRATIONS AND PERCENT REMOVAL AT SECONDARY TREATMENT PLANTS

Location	Sample	SON, mg/l			SON, % Removal		SON/SCOD			
		Infl.	Primary Effl.	Secondary Effl.	Primary Treat.	Prim.+ Second. Treat.	Infl.	Primary Effl.	Secondary Effl.	Average
Union City #3	1	4.1	3.5	1.1	15	73	0.043	0.039	0.031	0.037
	2	6.3	4.9	1.9	22	73	0.040	0.040	0.037	0.039
Palo Alto	1	5.5	4.4	2.1	20	62	0.060	0.058	0.059	0.058
	2	4.9	4.4	1.2	10	76	0.050	0.046	0.062	0.056
San Jose/ St.Clara	1	4.6	4.1	1.5	11	67	0.022	0.021	0.044	0.032
	2	6.3	5.5	1.8	13	71	0.024	0.023	0.046	0.036
Tahoe	1	2.9	-	1.2	-	59	0.024	-	0.046	0.041
Average		4.9	4.5	1.5	15	69	0.038	0.038	0.046	0.043

waste origin, flow rate, mixed liquor suspended solids (MLSS), and loading at the several plants. No trends have been found in the composite sample data which might indicate the importance of a particular parameter in the removal of SON by secondary treatment. An estimate of the relative proportion of nitrogen contained in the soluble organics can be obtained by taking the ratio of SON to SCOD. SON to SCOD ratios varied widely among the treatment plants sampled, seemingly a function of raw waste characteristics, but were quite constant at an individual plant, as shown in Table 12.

DIURNAL VARIATION IN SON

At three of the treatment plants sampled (Palo Alto, San Jose/Santa Clara and Union City) grab samples were taken every two hours and analyzed for SON, SCOD, and ammonia. Figures 1 through 3 show the diurnal variations in SON after various processes in the three treatment plants. Significant differences exist in the patterns in the influent for the three plants, but no explanation for the differences can be given. Ratios of maximum to minimum influent SON varied from a high of 4.5 at the Union City plant to a low of 2.0 at Palo Alto, and ratios of maximum to average SON varied from 1.9 at the San Jose/Santa Clara plant to 1.2 at the Palo Alto plant. Variations in effluent SON were much smaller, maximum to minimum values ranging from 1.7 to 1.3, and maximum to average ratios ranging from 1.3 to 1.1.

In all cases there was a close relationship between SON and SCOD. Variations in ammonia and SON were sometimes similar, but not as similar as SON and SCOD.

An interesting pattern was noted in the SON data from the Union City plant. The influent SON concentration appeared to be reflected 5 to 6 hours later in the effluent as shown in Figure 4, which is taken from Figure 3. This pattern may indicate that a certain portion of the influent SON resisted treatment.

PALO ALTO SECONDARY EFFLUENT

A more detailed study of the organic and inorganic constituents of Palo Alto Secondary Effluent (PASE) was made, since this effluent was used for each of the experiments to determine the potential of various processes for removal of SON. Table 13 presents the results of a six-month study of the general characteristics of composite samples of PASE.

A sample of PASE was taken on February 6, 1976, filtered, and analyzed for various organic constituents for comparison with the results of other investigators. The sample had an SON concentration of 1.67 mg/l and an SCOD concentration of 28 mg/l. The concentration of MBAS was 0.08 mg/l as linear alkylate sulfonate (LAS); carbohydrates were 1.7 mg/l as glucose; and hydroxylated aromatics were 1.3 mg/l as tannic acid. MBAS was present in much smaller amounts than reported for other secondary effluents [22,23,26], while carbohydrate and hydroxylated aromatics concentrations were typical.

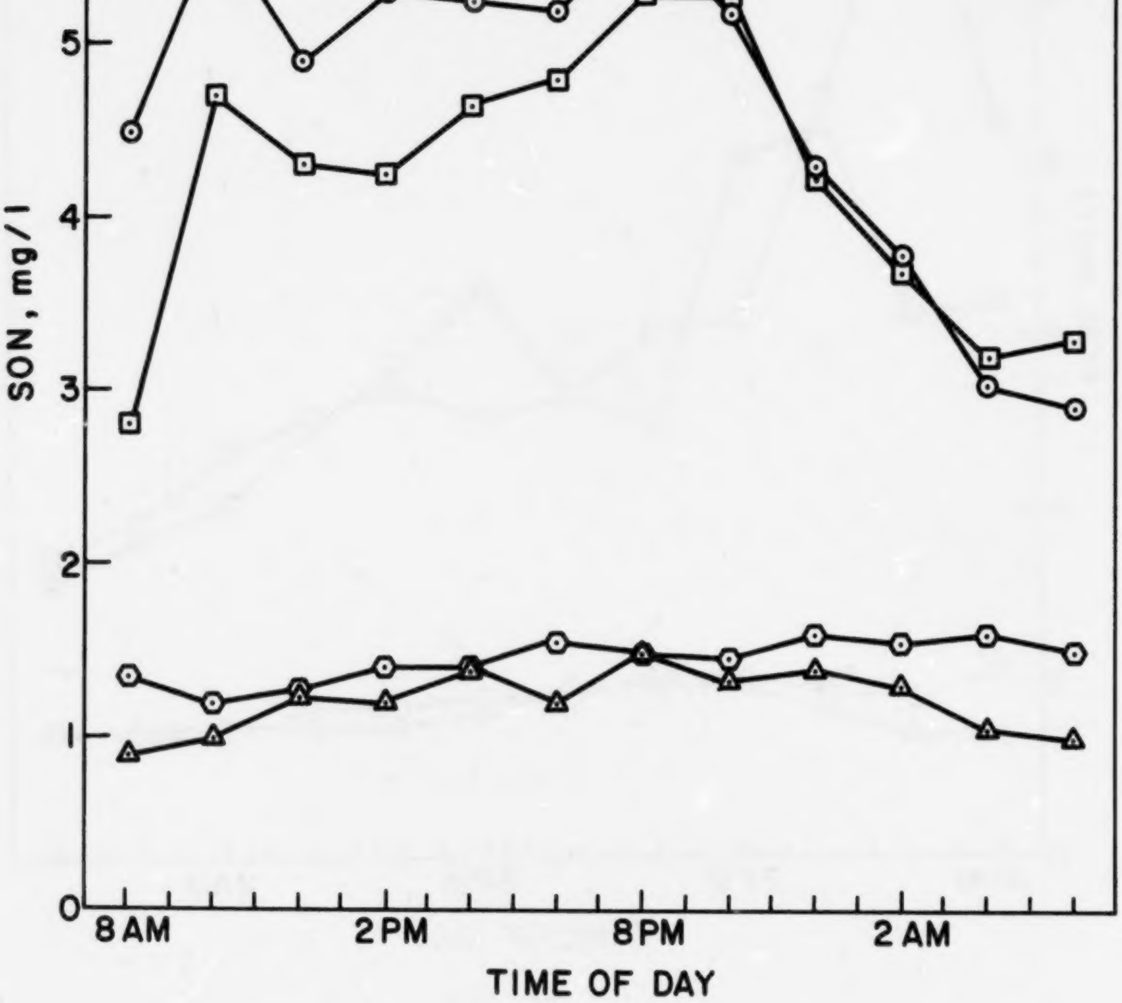


Figure 1. Diurnal variation of SON -- Palo Alto (4-14-75).

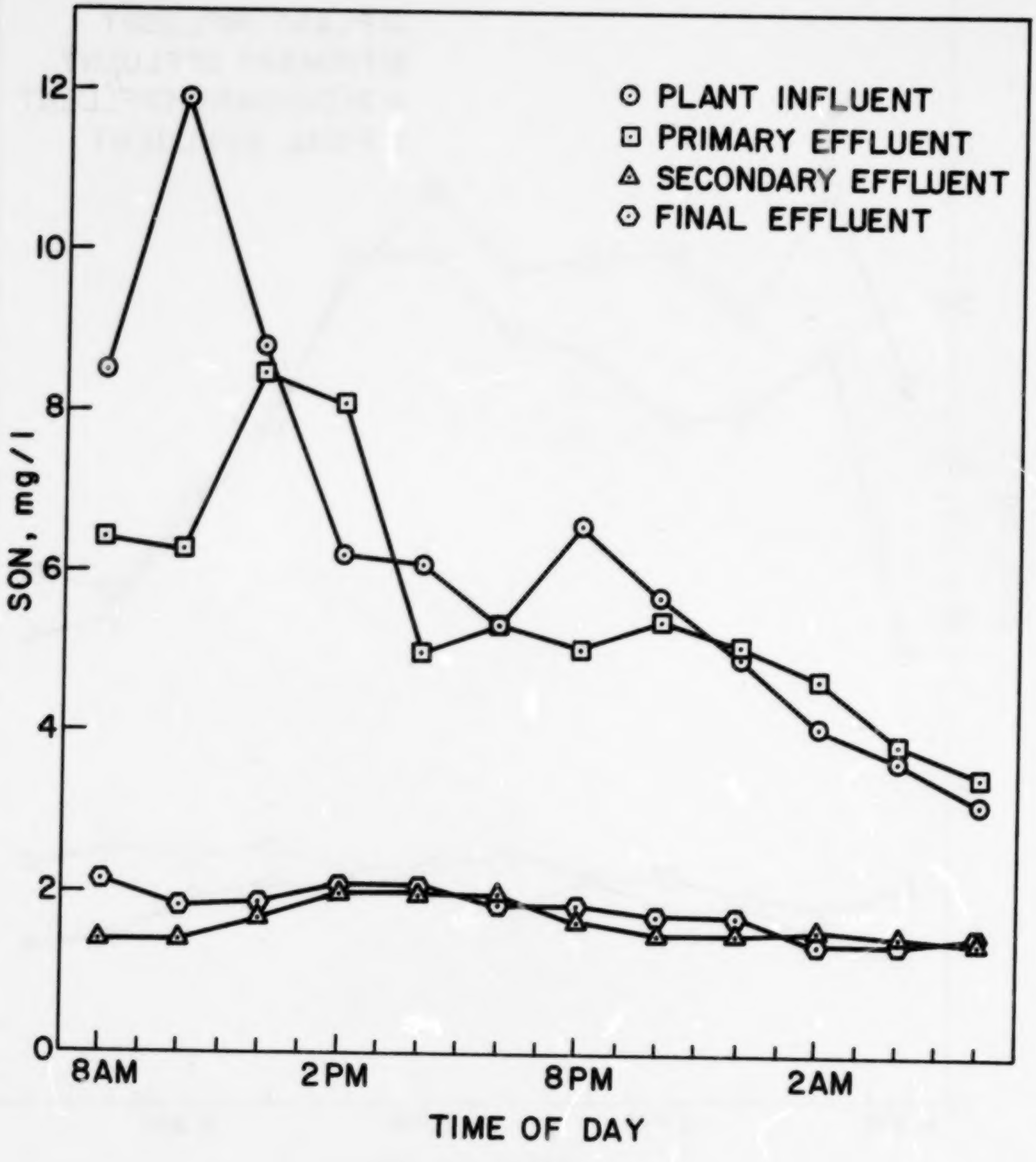


Figure 2. Diurnal variation of SON -- San Jose/Santa Clara (5-13-75).

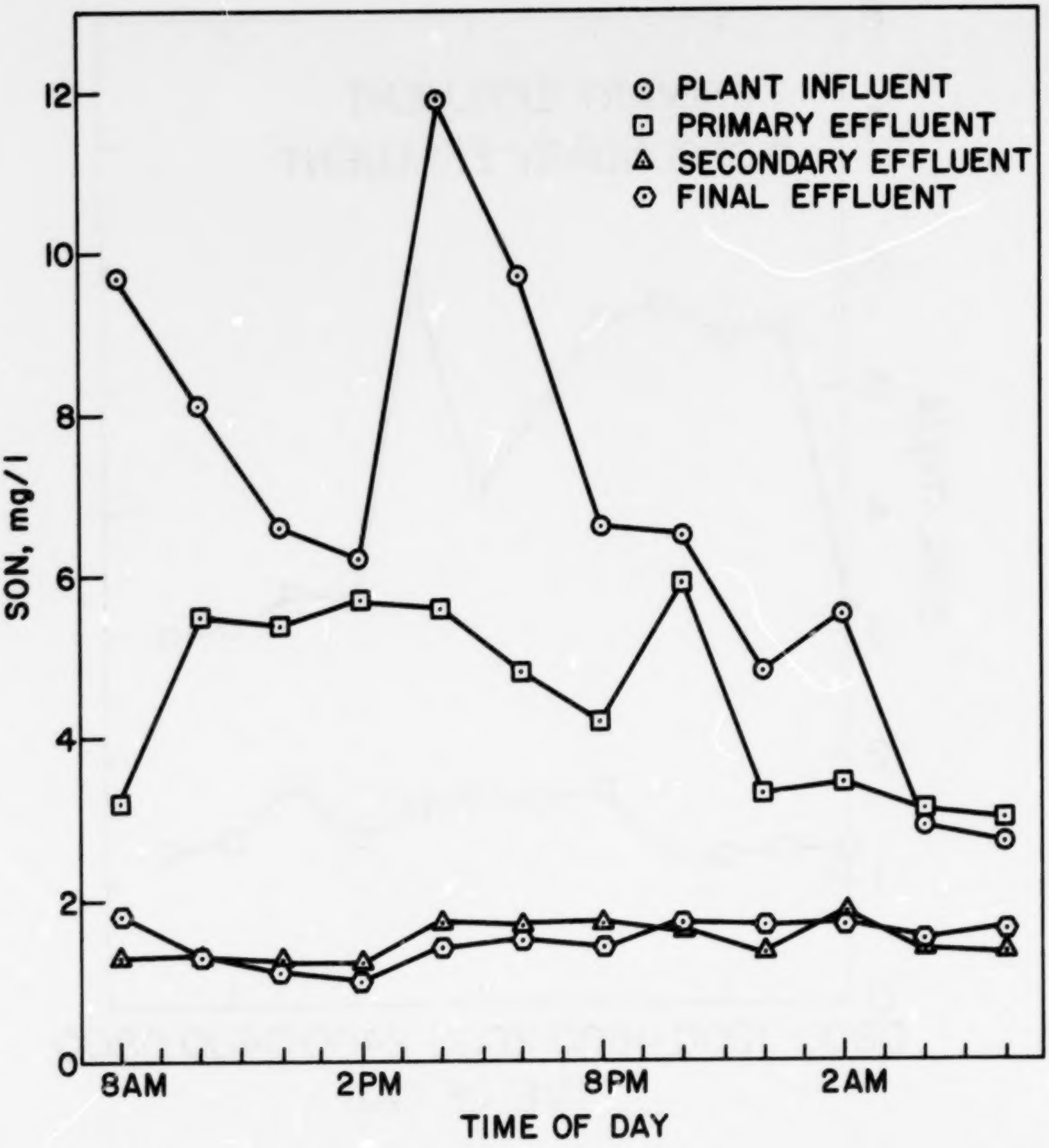


Figure 3. Diurnal variation of SON -- Union City #3 (5-1-75).

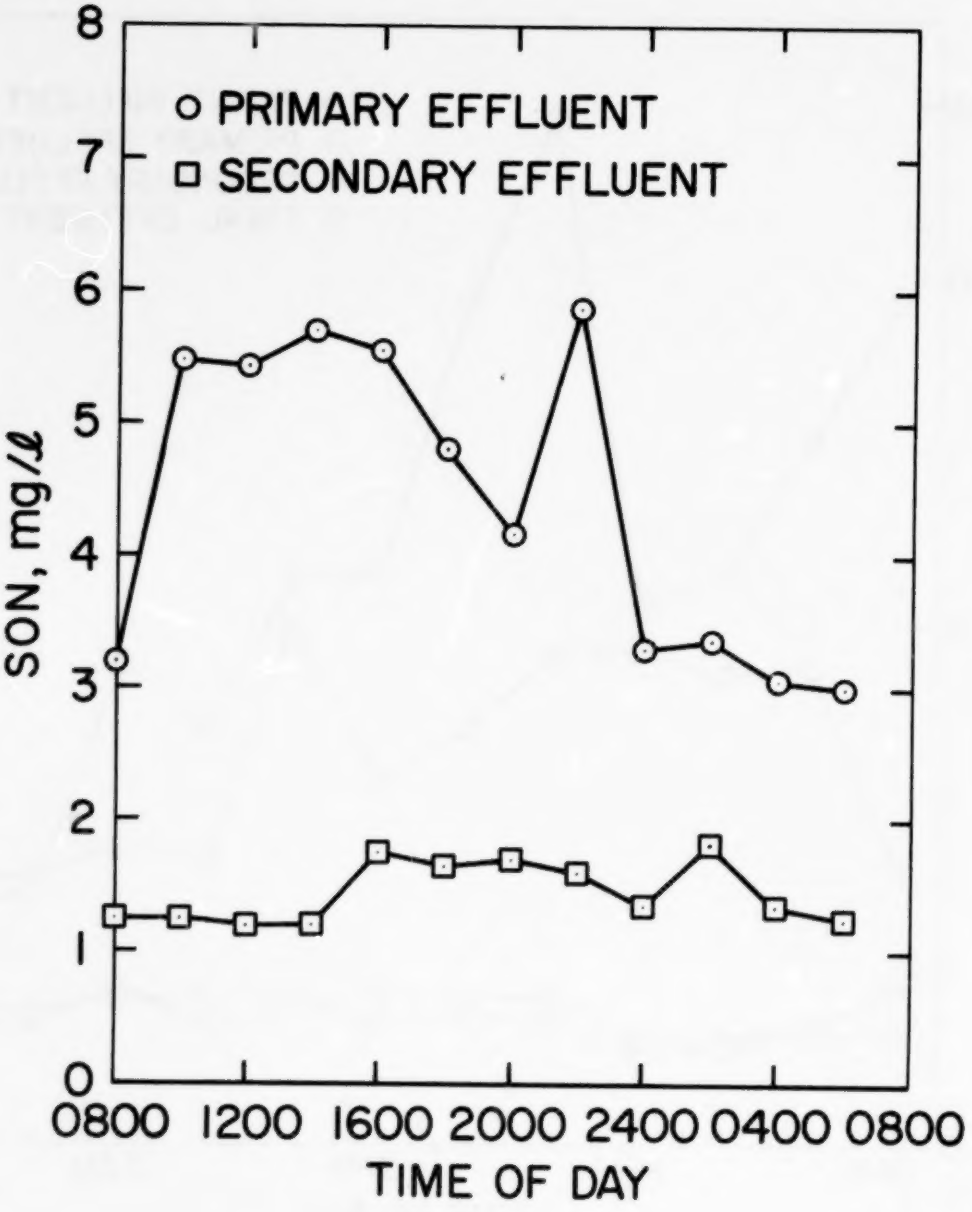


Figure 4. Diurnal variation of SON at Union City #3 (5-1-75), (taken from Figure 3).

TABLE 13. GENERAL CHARACTERISTICS OF PALO ALTO SECONDARY EFFLUENT*

Parameter	Mean Value	Standard Deviation	Minimum Value	Maximum Value	Number of Samples
SON, mg/l	1.29	0.24	0.84	1.66	20
SCOD, mg/l	28	4.8	20	39	20
Soluble BOD$_5$, mg/l	1.9	1.6	0.2	5.0	16
Organic-N, mg/l†	3.0	0.6	1.4	3.8	20
COD, mg/l†	53	41	25	166	17
BOD$_5$, mg/l†	12	5.3	6	28	18
TOC, mg/l†	22	7.2	13	36	19
pH	7.6**	-	7.4	8.1	20
Suspended solids, mg/l†	16	5	7	28	19
Nitrate-N, mg/l	0.4	0.7	0.0	3.1	20
Nitrite-N, mg/l	0.3	0.6	0.0	2.4	20
NH$_3$-N, mg/l	24	4.8	17	35	20

*Based upon 24-hour composite samples taken between June 11, 1975 and December 13, 1975.

†Values obtained from Palo Alto Regional Water Quality Control Plant Records.

**Median.

The protein analysis was of particular interest, since (1) protein is a possible constituent of SON; (2) various investigators have reported its presence in secondary effluent [22,23,26]; and (3) it is generally considered to be highly biodegradable. The PASE sample described above was analyzed for protein using the technique of Lowry et al. [27], using ovalbumin and gelatin protein standards, and the protein concentration was found to be 8.0 mg/l by this method. The technique does not measure nucleic acid bases or urea, and amino acids give much less color than proteins; however, most phenols will interfere in the analysis. Close inspection of the Lowry method shows that it is virtually the same analysis as that for hydroxylated aromatics, the important exception being the presence of the copper catalyst. The copper has been demonstrated to have little effect on the color produced with tyrosine and tryptophan [27], which are hydroxylated aromatic compounds. When the copper catalyst was omitted from the analysis, the same concentration of "protein" in PASE was still measured by the test. This indicates that the color was produced by hydroxylated aromatics rather than protein, and that the concentration of protein was in fact negligible. It is believed that the sample did not contain sufficient copper to serve as a catalyst.

DeWalle and Chian [28] were probably correct in stating that fulvic acids interfere in the Lowry test by virtue of their numerous phenolic hydroxyl groups. The Lowry method is an adaptation of an earlier method of Folin and Ciocalteu [52] for measuring tyrosine and tryptophan, through the reaction of their phenolic groups with the Folin phenol reagent used in both the Lowry test and the test for hydroxylated aromatics.

SUMMARY

1. The concentration of SON in the secondary effluents of four activated-sludge treatment plants ranged from 1.1 to 2.1 mg/l, with an average of 1.5 mg/l.

2. The concentration of SON in the secondary effluent of one treatment plant ranged from 0.9 to 1.7 mg/l, with an average of 1.3 mg/l over a six-month period.

3. Diurnal variations in the concentration of SON in the secondary effluents of three treatment plants were small, with maximum to minimum ratios ranging from 1.7 to 1.3, and maximum to average ratios ranging from 1.3 to 1.1 mg/l.

4. Variations in SON and SCOD were very similar in all cases.

5. Data from one treatment plant indicate that the effluent concentration of SON may be a function of the influent concentration; however, there was no other indication of the influence of any one factor on effluent SON concentration.

6. Little or no protein was found in a filtered sample of Palo Alto secondary effluent.

SECTION 7

SON REMOVAL BY PHYSICAL AND CHEMICAL PROCESSES

REMOVAL MECHANISMS

Removal of SON from municipal secondary effluent can potentially be accomplished through two general mechanisms: (1) physical removal mechanisms, resulting in physical removal of SON from solution; and (2) oxidative removal mechanisms, resulting in oxidation of the nitrogen atom or of the organic carbon, resulting in the release of ammonia.

As shown in Table 14, three mechanisms for physical removal of SON were evaluated in this study: (1) adsorption, (2) flocculation, and (3) precipitation. Adsorption is defined as the accumulation of a solute at the solid-liquid interface, and will be used here to include ion exchange and co-precipitation.

Flocculation is the aggregation of minute particles, whose surface charges have been sufficiently neutralized, into large flocs which can generally be removed from solution by sedimentation. Should any of the SON be in

TABLE 14. FACTORS AFFECTING REMOVAL AND REMOVAL MECHANISMS

FACTORS AFFECTING REMOVAL:

A. Charge (electrostatic interactions).

B. Polarity (dipole-dipole interactions, dipole-induced dipole interactions, van der Waals forces, pi-bond interactions, hydrogen bonding, solvation)

C. Special factors (e.g., covalent bonds, pore size, entrapment)

D. Chemical structure and reactivity.

REMOVAL MECHANISMS:

1. Adsorption (includes ion exchange and co-precipitation) -- (Factors A, B, and C)
2. Flocculation -- (Factors A, B, and C)
3. Precipitation -- (Factor C)
4. N-oxidation -- (Factor D)
5. Deamination -- (Factor D)

colloid form, it could be removed in this manner. Truly soluble SON can also be removed by flocculation if sufficient quantities of the flocculant adsorb onto an SON molecule to enable it to behave as a small colloid and flocculate with other particles.

Precipitation occurs in the special circumstance in which a covalent bond is formed between the surface and the solute or between an ion and the solute, such that the solute is rendered insoluble.

There are two mechanisms by which oxidation can occur: (1) N-oxidation, in which the nitrogen atom is oxidized to a higher oxidation state and is no longer measured by the Kjeldahl organic nitrogen analysis; and (2) C-oxidation, following which the nitrogen is released as ammonia. Removal of SON by oxidation may potentially be accomplished by either chemical or biological oxidation of the nitrogen atom. In either case, the nitrogen atom need not be "physically" removed from solution, but is chemically altered to either inorganic nitrogen or to a higher oxidation state of organically-bound nitrogen, such that it is no longer measured by the Kjeldahl organic nitrogen analysis. Nitrogen thus oxidized will be termed "removed," even though the nitrogen atom is still present in solution. The ultimate removal, effects, and characteristics of the chemically altered compounds produced by oxidation were beyond the scope of this study.

There are a number of operating parameters such as pH, surface area, contact time, and temperature, which affect the overall efficiency of a treatment process. These parameters will be discussed along with each process. The following discussion will focus on the specific factors which are important in determining whether a particular molecule can potentially be removed by a particular removal mechanism.

The factors affecting removal have been arbitrarily grouped into four categories: (1) charge, (2) polarity, (3) chemical structure and reactivity, and (4) special factors. Charge affects the physical removal of a solute through attractive and repulsive electrostatic interactions. A molecule bearing the same charge as a surface has less chance of being adsorbed to that surface than a molecule of the opposite charge. However, adsorption of a molecule onto a surface of like charge is possible, provided that other factors affecting removal are sufficiently favorable.

Polarity is the term under which the following factors will be grouped: dipole-dipole interactions, dipole-induced dipole interactions, van der Waals forces, pi-bond interactions, hydrogen bonding, and solvation. All, with the exception of solvation, are generally considered to favor adsorption, but the forces involved are usually very small relative to electrostatic forces. Solvation relates to the energy required to displace water from the surface and from the hydration sphere surrounding the molecule, enabling the molecule and the surface to come in contact. Solvation always hinders adsorption.

There are a number of special factors related to the physical removal of a substrate, such as (1) the formation of a covalent bond between surface and substrate; (2) the pore size of the surface, which may be too small to admit a molecule that might otherwise adsorb; or (3) entrapment of a large molecule by floc particles. Such special factors can prevent the removal of a molecule that might otherwise be removed or cause a molecule to be removed from solution despite unfavorable conditions.

The primary factors affecting the removal of SON by oxidation are the chemical structure of the nitrogen-containing compounds and the reactivity of the various chemical bonds.

There are a number of advanced wastewater-treatment processes which are potentially capable of removing a significant fraction of SON from municipal secondary effluent. Those evaluated in this study are listed in Table 15 together with the removal mechanisms pertinent to each.

Each of the processes considered to have potential for the removal of SON has been studied extensively, and many models exist for each. However, the majority of the work has been carried out on model systems employing perhaps a single homogeneous substrate, a few ions of known concentration, and a well-characterized solid. Secondary effluent poses a far more complex problem in

TABLE 15. TREATMENT PROCESSES AND REMOVAL MECHANISMS

TREATMENT PROCESSES:

1. Chemical Coagulation -- (Mechanisms 1, 2, and 3)
    - i. Ferric Chloride
    - ii. Lime
    - iii. Alum
2. Adsorption -- (Mechanisms 1, 2, and 3)
    - i. Activated Carbon
    - ii. Ion-Exchange Resins
3. Oxidation -- (Mechanisms 4 and 5)
    - i. Chemical Oxidation
        - (a) ozone
        - (b) chlorine
        - (c) potassium permanganate
        - (d) hydrogen peroxide
    - ii. Biological Oxidation

REMOVAL MECHANISMS:

1. Adsorption (includes ion exchange and co-precipitation)
2. Flocculation
3. Precipitation
4. N-Oxidation
5. Deamination

terms of (1) the substrate, and (2) the concentration and number of ions present.

The large number of different compounds included in an analysis for a general parameter such as SON adds complexity to interpretation of results from both physical and oxidative removal processes. In terms of the physical removal processes, a molecule of SON can be positively charged, negatively charged, uncharged, polar, non-polar, colloidal, soluble, or a combination of these. It may also possess some special characteristic which allows it to be removed by virtue of a special factor. In addition, certain organic molecules present may affect the removal of others by altering the properties of a surface or by complex formation.

In terms of the oxidative processes, the amount of SON existing in each particular configuration is not known, making it difficult to predict the reactions which will occur, the rate of individual reactions, and the end products of the reaction.

The numerous anions and cations present in secondary effluent add another level of complexity to the problem. The role of each ion in catalyzing or inhibiting oxidation is not known, nor is the degree to which the organic material complexes with different ions.

Due largely to the complex ionic makeup of secondary effluent, the properties of a surface introduced into the effluent may be considerably different from those of the same surface in a simpler system. The thickness and properties of the electrical double layer surrounding a charged surface are a function of the ionic characteristics of the surrounding liquid. Ions coprecipitated during chemical coagulation may alter such properties of the flocs as isoelectric point or sedimentation rate.

Thus, the ionic and chemical nature of secondary effluent presents a large number of variables, such that it is virtually impossible to examine each of them individually in a reasonable period of time. In addition, it becomes very difficult to distinguish between the various removal mechanisms and the factors affecting these mechanisms. The complexity of this system necessitates an approach somewhat different than generally used to investigate simpler systems.

EXPERIMENTAL APPROACH

The experiments for this phase of the study were conducted to determine:

1. The potential of various physical and chemical processes for the removal of SON and the factors affecting removal.
2. The potential of various combinations of treatment processes for removal of SON.

Removal of SON by Various Treatment Processes

This portion of the study was of use in meeting two of the objectives of the study: bench-scale studies allowed evaluation of the potential of advanced waste-treatment processes for SON removal, and investigation into the various factors affecting removal provided information useful in determining the nature and characteristics of the SON. The processes studied can be divided into three categories: (1) chemical coagulation, (2) adsorption, and (3) chemical oxidation. (Biological oxidation is discussed in Section 8.)

Chemical coagulation was evaluated with ferric chloride, lime, and alum as coagulants, together with various polyelectrolytes and clays as coagulant aids. Variables studied were pH, mixing time, and coagulant concentration.

Adsorption of SON was studied using granular activated carbon and ion-exchange resins as adsorbents and pH and adsorbent dose as variables. (Adsorption is used here in the sense of adsorption "process"; the adsorption "mechanism" is, of course, applicable to both adsorption processes and chemical coagulation.)

Chemical oxidation was studied using ozone, chlorine, hydrogen peroxide, and potassium permanganate as oxidants, and pH and oxidant dose as variables.

Due to the complexity of the systems under investigation, it was considered unreasonable to attempt to determine the effect of each different ion on the removal of SON by each process. Analysis of the Palo Alto effluent revealed that the major inorganic constituents of the wastewater remained relatively constant with time [51], and so variations in inorganic contents were not considered a significant factor affecting noted variations in results.

Removal of SON by Combinations of Processes

It is likely that the SON removed by one treatment process differs in some way from that removed by another, such that a combination of the two processes would be capable of removing a greater amount of SON than either individually. A number of bench-scale experiments were carried out on Palo Alto secondary effluent. The processes selected for these experiments were generally those which were thought to have the greatest possibility for removing different fractions of the SON, although at times, combinations of processes thought to be removing the same fraction were tested to see if this was in fact the case.

In contrast to the case with individual treatment processes, it was not possible in these experiments to consider that the inorganic ion concentrations were constant. A number of the treatment processes, such as ion exchange or chemical coagulation, are capable of radically altering the inorganic characteristics of a secondary effluent. This greatly enhanced the possibility that one treatment process might hinder or improve removal by a second process. This necessitated very careful consideration of the possible effects of inorganic ions on the experimental results.

Background

The coagulants commonly used for water treatment and advanced wastewater treatment include aluminum sulfate (alum), ferric chloride, ferric sulfate, calcium oxide (lime), and magnesium oxide. In this study, alum, ferric chloride, and lime were evaluated for their potential to remove SON from PASE.

A number of "coagulant aids" are commercially available which can theoretically improve coagulation in various ways, such as bridging floc particles, neutralizing charge, or providing nuclei for flocculation. Polyelectrolytes, polymers containing positively and/or negatively charged functional groups; clay minerals, such as bentonite, koalinite, and montmorillonite; activated silica; and activated alumina are some of the materials used as coagulant aids. The properties and behavior of polyelectrolytes have been discussed in detail by LaMer and Healy [70], Ries and Meyers [71], Black [72], and Stumm and O'Melia [53]. Several coagulant aids were evaluated in this study for their potential to improve SON removal.

Chemical coagulation is a process in which the surface charge of colloidal particles is reduced through adsorption of the coagulating ions or their hydrolysis products. The particles may then be flocculated and removed by sedimentation. Under conditions of sufficient dose and proper pH, coagulants will form a precipitate, which provides centers for flocculation, and which is potentially capable of removing soluble organics by adsorption.

In the treatment of wastewaters, chemical coagulation has been employed for the removal of suspended solids following secondary effluent and for the treatment of kraft mill effluents and other industrial wastewaters. Optimization for the efficient removal of organic materials has been largely oriented toward removal of the particulate fraction, with relatively little attention given to the parameters of possible importance in removing the soluble fraction of the organics.

Parkin and McCarty [73] studied the removal of SON from a treated agricultural wastewater with various coagulants and polyelectrolytes in combination with bentoni[illegible]. They found that ferric chloride achieved about 67 percent removal o[illegible] compared with 43 percent for lime, and 35 percent for alum. Polyelectr[illegible]s achieved only about 16 percent removal at neutral pH, but removal was improved at pH 3 and 11, possibly due to ionization and precipitation of SON, respectively.

Malhotra et al. [74] were able to remove 60 percent of the total organic nitrogen and 55 percent of the COD from a secondary effluent using 250 mg/l of alum at pH 5, the average initial organic nitrogen concentration being 4.3 mg/l and the COD 91 mg/l. Wolf [75] reported an average organic nitrogen removal from a trickling filter effluent of about 30 percent using iron salts and lime. Rebhun et al. [76] studied the effects of polyelectrolytes and bentonite clay on removal of organic contaminants, and found that bentonite removed about 50 percent of the total organic nitrogen at a dose of 500 mg/l; however, the initial concentration of organic nitrogen was 14.0 mg/l. In most cases for which data are available, it appears that organic nitrogen removal by chemical coagulation closely parallels COD removal. The soluble portion of organic nitrogen and COD removed in these experiments was not indicated.

Assuming some portion of the soluble organics are removable by coagulation, the primary mechanism would be adsorption. The factors which together determine whether a particular molecule will adsorb can be grouped into three categories: (1) charge, (2) polarity, and (3) special factors.

Charge has received the greatest amount of attention in the literature for several reasons: (1) it is the most important factor in the removal of particulate and colloidal matter; (2) it is relatively well understood; (3) a number of models exist which deal with the nature and properties of charge and the parameters affecting it; and (4) there are a number of simple techniques available for studying charge including electrophoresis, titration, maximum sedimentation rate determination, and surfactant adsorption [77,78].

When a coagulant is added to water under conditions such that a precipitate forms, the hydrous oxide surface of the precipitate can be positively or negatively charged, depending on the pH and a number of other factors. The pH at which the net charge on the surface is zero is referred to as the point of zero charge or PZC.

The factors controlling the sign and magnitude of the surface charge on oxides have been discussed in detail by Parks [79,80]. Hydrous oxides behave as ion exchangers and can adsorb ions from solution which may considerably alter the surface charge. For example, a calcium hydroxide precipitate will be positively charged at pH 11 in deionized water, but even small amounts of phosphate or bicarbonate can reverse this charge [81]. In general, anions will lower the PZC if adsorbed, and cations will shift the PZC toward the PZC of each individual cation.

The particulate matter found in secondary effluent is negatively charged [82], and it is generally accepted that the large majority of the soluble contaminants are also negatively charged. If true, optimum removal of organics would seem to call for a positively charged surface. If charge is in fact an important factor, and this has yet to be established, then conditions which tend to make the surface charge of a floc more positive should result in better removal. If such conditions also make the organics positive, then the potentially better removal may be canceled.

Polarity is a factor that is not as well understood or often modeled as charge, and it is somewhat controversial in several respects. The hydrogen-bonding mechanism can explain the removal of certain molecules from solution, but water itself is a strong hydrogen-bonding solvent. Solutes can be described as either hydrophilic or hydrophobic, but there is not a distinct boundary between the two. In the case of chemical coagulation, the surfaces involved are polar oxides; hence it is not likely that polarity plays a major role in determining adsorption due to relatively small differences in polarity between surface and solvent.

Special factors are likely to dominate in the adsorption of soluble organics during chemical coagulation. The definition of soluble used in this study is an operational one, and it is probable that a fraction of the "soluble" organics is in fact comprised of very small colloids. These colloids, as well as some of the larger molecules in solution, may be removed by entrapment in the coagulant floc. Specific adsorption and precipitation are also likely to be of importance.

Variables of importance in the chemical coagulation process are pH, temperature, ionic strength, coagulant dose, and mixing time. Since ionic strength and temperature cannot be conveniently modified under treatment plant conditions, they were treated as constants in this study, and pH, mixing time, and coagulant dose were the variables studied.

The pH at which samples are coagulated is an important variable, since it will determine the charge of the organics being removed, the nature of the coagulant metal hydrolysis products, and the surface charge of the resultant floc particles. Malhotra et al. [74] found the optimum pH for alum coagulation of phosphorus to be 5, and pH 6 to be superior to pH 8 for organic nitrogen removal. The optimum pH for removal of negative colloids usually falls in the range of 5 to 6.5 [83].

Experimental Procedures

The coagulation experiments were carried out to determine (1) the potential of alum, lime, and ferric chloride for removing SON; (2) the various parameters affecting removal; (3) the effect of coagulant aids on removal; and (4) the reasons for any differences in removal among the various coagulants.

Samples of PASE were taken between 11:00 AM and 3:00 PM and brought to the laboratory for immediate use, and were not filtered unless otherwise indicated. Filtration prior to coagulation was conducted with a Pall filter, while filtration after coagulation was with a Millipore filter. The majority of the samples were not filtered prior to coagulation in order to simulate actual treatment plant conditions, and it was determined that filtration resulted in only small differences in final effluent quality. For those experiments involving activated sludge, the activated sludge was taken from an aeration tank at the Palo Alto plant and used immediately without prior filtration.

One to two liters of sample were placed in a beaker and stirred at 100 rpm with a 6-place stirrer (Phipps and Bird) while the coagulants and other reagents were added, all chemicals being at least reagent grade. The ferric chloride and alum coagulants were added from stock solutions of 100-200 mg/l, and lime and the clays were added as slurries containing 50-200 mg/l. The desired pH was obtained by addition of sodium hydroxide (1 to 6 N). All experiments were carried out at room temperature, approximately 20°C.

The polyelectrolytes were obtained from the Dow Chemical Company in their solid or most concentrated form and diluted to suitable working solutions. Those used were a cationic polymer, C-31; an anionic, A-23; and a non-ionic, N-17.

Mixing time was 20 minutes at 25 rpm and settling time was 30 minutes unless otherwise noted, after which the supernatant liquid was siphoned off and analyzed for SON, SCOD, pH, and turbidity.

Control samples underwent the same procedures but with no coagulant added. Values of SON and SCOD for the control samples were determined on their filtrate through a 0.45 micron filter (Millipore).

In the jar tests involving the clay minerals, kaolinite (J. T. Baker, Kaolin, technical grade) or bentonite (Wyoming, 325 mesh, Wards Scientific) was added to the sample while stirring at 100 rpm, and after 5 minutes, the coagulant was added in the usual manner.

In experiments to determine the effect of coagulant dose on SON removal, with alum and ferric chloride, the pH was adjusted to between 5 and 6.5. Fine adjustments of the pH with these coagulants was hindered by the fact that they "age" by adsorbing base, which causes a drift in the pH during the course of the experiment. The pH values recorded were those of the supernatant liquor after settling.

CHEMICAL COAGULATION

Coagulation of PASE Samples

It was desirable to know if the organics remaining after coagulation were entirely in the soluble fraction, or if there were still particulate matter in the samples. Therefore, three samples of secondary effluent were coagulated with a small dose of each of three coagulants, and both filtered (0.45 micron Millipore) and unfiltered portions of the supernatant liquid were analyzed for organic nitrogen and COD. The results (Table 16) show that doses of 200 mg/l or less of the three coagulants removed virtually 100 percent of the particulate organic matter.

Thus, coagulated samples do not require filtration prior to analysis for soluble constituents. For coagulated samples, then, total organic nitrogen is assumed equivalent to SON.

TABLE 16. COMPARISON BETWEEN FILTERED AND UNFILTERED COAGULATED PASE SAMPLES

Coagulant	Coagulant dose, mg/l	Organic Nitrogen, mg/l		COD, mg/l	
		Total	Soluble	Total	Soluble
$FeCl_3$	200	0.75	0.73	16	16
CaO	200	0.89	0.91	17	18
$Al_2(SO_4)_3$	150	1.26	1.27	22	21

In most current advanced wastewater-treatment schemes, coagulation follows secondary clarification and precedes filtration. It would seem unreasonable to coagulate an effluent after removing the particulate matter by filtration. However, it is conceivable that the presence of particulates could influence the removal of soluble material, perhaps by making the floc particles more negatively charged or acting as a coagulant aids. To test this hypothesis, both filtered and unfiltered portions of a sample of PASE were coagulated with lime and with ferric chloride. The results, presented in Table 17, indicate that only very small (but significant) differences in removal of SON resulted from filtration prior to coagulation.

TABLE 17. COAGULATION OF FILTERED AND UNFILTERED PASE SAMPLES

Sample	Coagulant	pH	SON, mg/l	Percent SON Removed	SCOD mg/l	Percent SCOD Removed
Control	--	7.7	1.20	-	24	-
Filtered	600 mg/l $FeCl_3$	6.1	0.95	21	16	36
Unfiltered	600 mg/l $FeCl_3$	6.1	0.84	30	15	39
Filtered	400 mg/l CaO	11.4	0.76	37	17	32
Unfiltered	400 mg/l CaO	11.4	0.84	30	16	34

Mixing time is an important variable in chemical coagulation, both economically and operationally. In this study, it was desirable to have a mixing time long enough to ensure maximum removal of the soluble organics. Table 18 shows that with both ferric chloride and lime, maximum removal was achieved with a 15-minute mixing time. To ensure that mixing time was adequate in the remainder of the experiments, 20 minutes was selected.

In those experiments involving ferric chloride and alum, the coagulant was added first, depressing the pH sharply, and then sodium hydroxide was added to attain the desired pH. Such additions can create very high and low localized pH values, perhaps sufficient to alter the organics present, or to solubilize some of the particulate matter. An experiment in which a sample was coagulated both with precombined and separately added reagents showed identical results for both techniques. Thus, localized extreme pH concentrations of the magnitude generated in these experiments should not have affected the outcome of the experiments.

The coagulation of two samples with ferric chloride was studied as a function of pH to determine the optimum pH for removal of SON. These results are presented in Table 19 and indicate that pH 5 to 8 is the optimum range for SON removal by ferric chloride. The pH range 5 to 6.5 is optimum for removal of negatively charged colloids in general [83]. SON removals were relatively constant in the pH range 5 to 6.5, and this range was used in most of the remaining experiments.

TABLE 18. SON REMOVAL AS A FUNCTION OF MIXING TIME AT 25 RPM

Coagulant	Dose, mg/l	Mixing Time, minutes	SON, mg/l	Percent SON Removed
None	-	-	1.09	-
$FeCl_3$	600	5	0.76	30
$FeCl_3$	600	10	0.76	30
$FeCl_3$	600	15	0.66	39
$FeCl_3$	600	20	0.66	39
CaO	400	5	0.85	22
CaO	400	10	0.79	28
CaO	400	15	0.75	31
CaO	400	20	0.79	28

TABLE 19. EFFECT OF pH ON FERRIC CHLORIDE COAGULATION OF SON*

	pH	SON, mg/l	Percent SON Removed
Sample 1	4.5	0.90	33
SON = 1.35 mg/l	5.0	0.80	41
(> 6%, > 8%)**	5.5	0.79	41
	6.0	0.81	40
Sample 2	6.0	0.48	49
SON = 0.95 mg/l	7.0	0.56	41
(> 8%, > 12%)**	8.0	0.52	45
	9.0	0.60	37
	10.0	0.61	36

*Coagulant dose = 600 mg/l $FeCl_3$.

**Differences in percent removal to be exceeded for 95% and 99% significance level, respectively.

An optimum pH was not determined for alum coagulation, and it was assumed to be the same as for ferric chloride coagulation. Malhotra et al. [74] found the optimum pH for alum coagulation of phosphate to be 5.6 and that organic nitrogen removal was better at pH 6 than at pH 8.

Series of jar tests were conducted to determine the optimum coagulant dose for maximum SON removal by ferric chloride, alum, and lime. Results are listed in Tables 20, 21, and 22, respectively. In all cases, a coagulant dose of about 200-300 mg/l effectively removed as much of the SON and SCOD as higher doses.

Polyelectrolytes N-17, A-23, and C-31 (Dow Chemical Company) were tested as coagulants, but due to their high solubility and the nature of secondary effluent they failed to produce a settleable floc at concentrations up to 50 mg/l. As coagulant aids, they were tested in combination with lime and ferric chloride, and in all cases worked extremely well and produced a very clear supernatant liquor within minutes after stirring was stopped. However, they showed no potential for increasing the removal of SON with ferric chloride or lime, and actually increased the concentration of SON and SCOD (the polyelectrolyte contained organic nitrogen) unless used in very low doses.

Clay minerals possess a relatively large negative surface charge at neutral pH, and are frequently employed as coagulant aids. Although soluble organic contaminants in general are also most likely to be charged negatively, molecules containing organic nitrogen may have localized centers of positive charge which would allow them to adsorb on a clay mineral surface.

Koalinite was used as a coagulant aid in ferric chloride coagulation of PASE but did not increase SON removal. Bentonite, which has a much larger surface area than kaolinite, was used in combination with ferric chloride and C-31 and caused a substantially significant (99% CI) increase in SON removal from PASE of about 10 percent with a dose of 200 mg/l bentonite. It is possible that the bentonite merely increased the efficiency of flocculation, but this effect was not found with kaolinite nor with any of the polyelectrolytes. Thus, there appears to be a very small fraction of SON which is adsorbed by bentonite, but not removed by ferric chloride. Bentonite and C-31 together achieved an SON removal of 14 percent and a SCOD removal of 27 percent.

The differences in SON and SCOD removal, achieved by the three coagulants shown in Table 23, were tested for significance as described in Appendix A, Section 5. Ferric chloride removed significantly (99% CI) more SON than did lime, which in turn removed significantly (95% CI) more SON than did alum. This is the same order of effectiveness noted by Parkin and McCarty [73] for removal of SON from treated agricultural wastewaters; however, there is insufficient data to generalize this finding and extrapolate it to all biologically treated effluents.

There was not a significant difference between the SCOD removals achieved by the three coagulants, nor did alum remove significantly more SCOD than SON. However, ferric chloride removed significantly (99% CI) more SON than SCOD, and lime also removed significantly (95% CI) more SON than SCOD.

TABLE 20. SON AND SCOD REMOVAL BY FERRIC CHLORIDE COAGULATION

	$FeCl_3$, mg/l	pH*	Turbidity NTU	SON, mg/l	Percent SON Removed	SCOD,** mg/l	Percent SCOD Removed
Sample 1	0	7.2	-	1.06	-	23	-
	100	6.7	-	0.87	18	19	16
Organic Nitrogen = 1.4 mg/l	200	5.7	-	0.75	29	16	31
	250	3.6	-	0.70	34	16	32
COD = 29 mg/l	300	3.4	-	0.72	32	16	31
Alkalinity = 180 mg/l	350	-	-	0.81	24	19	19
	400	-	-	0.95	10	22	4
(9-24-75, 1:30 PM)	500	-	-	1.17	0	23	0
Sample 2	0	7.6	3.4	1.11	-	22	-
	100	6.5	1.1	1.03	7	17	22
Organic Nitrogen = 1.9 mg/l	200	5.5	0.8	0.91	18	14	37
COD = 31 mg/l	300	5.5	0.7	0.80	28	13	41
	400	5.2	1.2	0.86	23	14	37
Alkalinity = 192 mg/l	500	5.5	1.0	0.78	30	14	35
(9-26-75, 1:00 PM)	600	5.6	1.0	0.82	26	12	45
	700	5.7	1.4	0.78	30	14	37

*No base added in coagulation of Sample 1; adjusted with NaOH in Sample 2.

**Two aliquots analyzed for each sample.

TABLE 21. SON AND SCOD REMOVAL BY ALUM COAGULATION

	$Al_2(SO_4)_3$, mg/1	pH*	Turbidity, NTU	SON, mg/1	Percent SON Removed	SCOD** mg/1	Percent SCOD Removed
	0	7.5	5.0	1.65	-	24	-
Sample 1	100	7.0	1.2	1.52	8	24	2
	150	6.8	1.0	1.26	24	22	7
Organic Nitrogen = 2.7 mg/1	200	6.6	0.80	1.22	26	21	11
	250	6.4	0.80	1.21	27	20	18
COD = 40 mg/1	300	6.3	0.80	1.20	27	19	21
Alkalinity = 210 mg/1	350	6.1	0.65	1.21	27	18	23
	400	5.9	0.70	1.11	33	18	23
(9-9-75, 2:30 PM)	450	5.7	0.75	1.08	35	18	26
	500	5.5	1.2	1.13	32	19	21
	0	7.4	3.2	1.16	-	21	-
Sample 2	200	5.8	1.3	1.03	11	13	39
	300	5.9	0.60	0.85	27	13	38
Organic Nitrogen = 1.9 mg/1	400	6.0	0.50	0.83	28	11	46
	500	6.1	0.45	0.89	23	12	41
COD = 27 mg/1	600	6.1	0.40	0.86	26	13	37
Alkalinity = 192 mg/1	800	6.1	0.45	0.84	28	13	35
(9-29-75, 1:00 PM)	1000	6.2	0.40	0.84	28	13	39
	1200	6.2	0.35	0.93	20	13	38

*No base added in coagulation of Sample 1; adjusted with NaOH in Sample 2.

**Two aliquots analyzed for each sample.

TABLE 22. SON AND SCOD REMOVAL BY LIME COAGULATION

	CaO, mg/l	pH*	Turbidity NTU	SON, mg/l	Percent SON Removed	SCOD,** mg/l	Percent SCOD Removed
Sample 1 Organic Nitrogen = 1.4 mg/l COD = 25 mg/l Alkalinity = 216 mg/l (10-8-75, 1:00 PM)	0	7.6	2.2	1.05	-	20	-
	100	9.6	3.1	0.97	8	19	6
	200	10.4	1.8	0.89	15	17	18
	300	11.2	0.60	0.87	17	16	23
	400	11.6	0.50	0.79	25	17	18
	500	11.8	0.90	0.82	22	15	25
	600	11.9	0.85	0.73	30	13	37
	700	11.9	0.65	0.73	30	15	28
Sample 2 Organic Nitrogen = 1.4 mg/l COD = 28 mg/l Alkalinity = 154 mg/l (10-14-75, 12 noon)	0	7.3	1.80	1.12	-	23	-
	400	11.6	0.55	0.78	30	18	22
	600	11.9	0.50	0.73	35	19	16
	800	12.1	0.45	0.81	28	16	29
	1000	12.2	0.50	0.76	32	20	14
	1200	12.3	0.55	0.70	37	18	20
	1400	12.3	0.55	0.68	39	16	29
	1600	12.3	0.65	0.68	39	16	29
	1800	12.3	0.80	0.68	39	16	29

*pH not adjusted after lime addition.

**Two aliquots analyzed for each sample.

TABLE 23. COMPARISON OF SON AND SCOD REMOVAL BY FERRIC CHLORIDE, LIME, AND ALUM COAGULATION

Sample	Initial SON, mg/1	Initial SCOD, mg/1	$FeCl_3$			CaO			$Al_2(SO_4)_3$		
			Dose, mg/1	Percent SON Removed	Percent SCOD Removed	Dose, mg/1	Percent SON Removed	Percent SCOD Removed	Dose, mg/1	Percent SON Removed	Percent SCOD Removed
1	1.19	21	1000	48	19	1200	39	21	1000	23	17
2	1.25	-	1000	46	-	1000	30	-	1000	29	-
3	1.26	-	1000	44	-	1200	38	-	1000	24	-
4	1.43	25	600	43	39	400	30	36	-	-	-
5	1.19	24	600	44	29	400	26	22	-	-	-
6	1.18	23	600	36	27	400	31	29	-	-	-
7	1.09	23	600	39	31	400	31	14	-	-	-
8	1.00	-	600	39	-	600	40	-	-	-	-
9	1.06	-	600	47	-	600	36	-	-	-	-
10	1.06	23	250	34	32	-	-	-	-	-	-
11	1.35	27	600	40	39	-	-	-	-	-	-
12	1.11	22	500	27	39	-	-	-	-	-	-
13	1.40	27	600	45	29	-	-	-	-	-	-
14	1.38	23	600	38	29	-	-	-	-	-	-
15	1.35	21	600	44	38	-	-	-	-	-	-
16	1.57	30	600	46	40	-	-	-	-	-	-
17	1.47	25	600	42	32	-	-	-	-	-	-
18	1.20	31	600	39	30	-	-	-	-	-	-
19	1.09	-	600	48	-	-	-	-	-	-	-
20	0.95	-	600	49	-	-	-	-	-	-	-
21	1.05	20	-	-	-	600	27	27	-	-	-
22	1.10	22	-	-	-	400	39	13	-	-	-
23	1.12	23	-	-	-	1000	35	24	-	-	-
24*	1.16	24	-	-	-	400	43	33	600	31	33
25	1.65	24	-	-	-	-	-	-	400	30	22
26	1.16	21	-	-	-	-	-	-	600	26	39
Average	1.23	24		42 ± 5	32 ± 6		34 ± 5	24 ± 8		27 ± 3	28 ± 10

*Filtered prior to coagulation.

It was shown earlier, in Table 19, that ferric chloride coagulation was significantly (99% CI) more efficient in removing SON at pH 6.0 than at pH 10.0, and the results of Table 23 indicate that there are differences in the removal efficiencies of the three coagulants. In order to more closely compare the SON removals achieved by each of the three coagulants, the supernatant liquor from a number of jar tests was coagulated a second or third time with a different coagulant or pH, as shown in Table 24.

Interestingly, an additional removal of SON was achieved in several instances (Table 25). After an initial coagulation with ferric chloride, a second coagulation with ferric chloride or alum produced no significant increase in SON removal, but a second coagulation with lime increased SON removal by 17% (99% CI). After an initial coagulation with lime, a second coagulation with lime increased SON removal by 16% (99% CI). Both ferric chloride and lime removed a significant (99% CI) amount of SON following an initial alum coagulation, but a second alum coagulation produced no increase in SON removal. Other combinations of coagulations showed that: (1) there is no significant difference in the removals achieved by lime and ferric chloride at pH 10.0; (2) ferric chloride coagulation at pH 10.0 removes a significant (99% CI) additional fraction of SON after an initial coagulation at pH 6.0; and (3) ferric chloride coagulation at pH 6.0 removes a significant (99% CI) additional fraction of SON after an initial coagulation at pH 10.0.

The differences in removal between the various coagulants and the additional removals achieved by a second coagulation are explainable in terms of the factors affecting adsorption. The surface charge of the ferric chloride floc in PASE is more positive at pH 6.0 than at pH 10.0 (Lengweiler et al. [88] have determined the PZC of iron oxides in dilute solutions to be 6.7.) Since coagulation with ferric chloride at pH 10.0 removed about 10-15 percent less SON than at pH 6.0, it appears that electrostatic charge may be of some importance. Furthermore, there is a small but significant fraction not removed at each pH which is removed with a second coagulation at the other pH (see Table 24, Samples 2 and 4).

Since the oxide surfaces involved are very polar as well as highly hydrated, it is unlikely that polarity plays a significant role in SON removal. Thus, SON removal by chemical coagulation is postulated to result from electrostatic attraction and special factors, i.e., entrapment, precipitation, and specific adsorption.

Aluminum sulfate flocs have shown to have a PZC of about 8.0 [54,90], and are thus likely to be positively charged at pH 6.0, as are ferric chloride flocs at the same pH. Therefore, the differences in removal of SON between ferric chloride and alum (see Tables 23 and 24) are considered to be due to special factors. Ferric ion is known to form stronger complexes with carboxylic acid groups, which can be present in secondary effluent in the form of amino acids, carbohydrates, and fulvic acid. The floc formed by ferric chloride differs from that formed by alum in its size and settling characteristics, such that it is conceivable that the two flocs may not be of equal efficiency in entrapping large molecules. Thus, the additional removal of SON achieved by ferric chloride as compared to alum (Table 24) is due to a

TABLE 24. EFFECT OF SEQUENTIAL COAGULATION ON SON REMOVAL

	First Coagulant	Dose, mg/l	pH	Second Coagulant	Dose, mg/l	pH	Third Coagulant	Dose, mg/l	pH	Percent SON Removed
Sample 1	$FeCl_3$	1000	6.6	-	-	-	-	-	-	46
SON = 1.25 mg/l	$FeCl_3$	1000	6.6	$FeCl_3$	1000	6.2	-	-	-	50
(12-8-75, 11:30 AM)	$FeCl_3$	1000	6.6	CaO	1000	12.3	-	-	-	63
	$FeCl_3$	1000	6.6	$Al_2(SO_4)_3$	1000	6.2	-	-	-	52
(> 6%, > 9%)*	CaO	1000	12.1	-	-	-	-	-	-	30
	CaO	1000	12.1	CaO	1500	12.4	-	-	-	46
	$Al_2(SO_4)_3$	1000	6.5	-	-	-	-	-	-	29
	$Al_2(SO_4)_3$	1000	6.5	$FeCl_3$	1000	7.3	-	-	-	44
	$Al_2(SO_4)_3$	1000	6.5	CaO	1500	12.3	-	-	-	51
	$Al_2(SO_4)_3$	1000	6.5	$Al_2(SO_4)_3$	1000	7.4	-	-	-	30
Sample 2	$FeCl_3$	600	6.0	-	-	-	-	-	-	49
SON = 0.95 mg/l	$FeCl_3$	600	6.0	$FeCl_3$	600	10.0	-	-	-	61
(12-15-75, 11:00 AM)	$FeCl_3$	600	10.0	-	-	-	-	-	-	36
(> 8%, > 12%)*	$FeCl_3$	600	10.0	$FeCl_3$	600	6.0	-	-	-	61
Sample 3	$FeCl_3$	600	6.0	-	-	-	-	-	-	39
SON = 1.00 mg/l	$FeCl_3$	600	10.0	-	-	-	-	-	-	34
	CaO	600	12.1	-	-	-	-	-	-	40
(12-16-75, 11:15 AM) (> 8%, > 11%)*	CaO	600	12.1	$FeCl_3$	600	10.0	-	-	-	43
Sample 4	$FeCl_3$	600	6.0	-	-	-	-	-	-	47
SON = 1.06 mg/l	$FeCl_3$	600	6.0	CaO	600	12.0	-	-	-	51
	$FeCl_3$	600	6.0	$FeCl_3$	600	10.0	-	-	-	58
(12-17-75, 11:00 AM)	$FeCl_3$	600	6.0	$FeCl_3$	600	10.0	CaO	600	12.4	57
(> 8%, > 10%)*	$FeCl_3$	600	10.0	-	-	-	-	-	-	25
	$FeCl_3$	600	10.0	$FeCl_3$	600	6.0	-	-	-	55

*Differences in percent SON removal to be exceeded for 95% and 99% significance, respectively.

TABLE 25. ADDITIONAL SON REMOVALS ACHIEVED BY SEQUENTIAL COAGULATION

Initial Coagulant	Subsequent Coagulant	Additional SON Removal, %	C.I. %
Significant increase in SON removal:			
Lime	Lime	16	99
Ferric chloride (pH 6.6)	Lime	17	99
Alum (pH 6.5)	Ferric chloride (pH 7.3)	15	99
Alum (pH 6.5)	Lime	22	99
Ferric chloride (pH 6.0)	Ferric chloride (pH 10.0)	12	95
Ferric chloride (pH 6.0)	Ferric chloride (pH 10.0)	11	99
Ferric chloride (pH 10.0)	Ferric chloride (pH 6.0)	25	99
Ferric chloride (pH 10.0)	Ferric chloride (pH 6.0)	30	99
No significant increase in SON removal:			
Ferric chloride (pH 6.6)	Ferric chloride (pH 6.2)		
Ferric chloride (pH 6.6)	Alum (pH 6.2)		
Alum (pH 6.5)	Alum (pH 7.4)		
Lime	Ferric chloride (pH 10.0)		

combination of specific adsorption and entrapment, but the data do not indicate which factor is the more important.

Lime forms a negatively charged floc in wastewater due to the presence of phosphate and bicarbonate anions [81]. Thus, it must remove SON primarily by virtue of special factors, since most of the organics are also negatively charged. The calcium salts of many carboxylic acids are highly insoluble, calcium forms strong complexes with the carboxylic acid group, and the dense, rapidly settling nature of the lime floc undoubtedly aids entrapment. Once again, the data do not allow distinction between these mechanisms.

A second coagulation of one sample with lime resulted in an additional 16 percent removal of SON (Sample 1, Table 24), suggesting the importance of electrostatic charge, since the second floc would be considerably less negatively charged than the first, due to the absence of phosphate and bicarbonate anions.

The data presented in Table 24 suggest: (1) that two small fractions of SON are present, one more positively charged and one more negatively charged, and the removal of each depends upon the charge of the floc particles;

(2) that there are fractions of SON capable of specifically adsorbing to the floc particles of each of the three coagulants; and (3) that there may be a fraction of SON, consisting of colloidal material, which is removed by all coagulants either through flocculation or entrapment.

Since each of the coagulants removes SON by a combination of electrostatic charge and special factors, it is conceivable that for different wastewaters, different coagulants may prove superior. Thus, for a particular wastewater, a series of jar tests would be appropriate for determining the optimum coagulant.

Direct Addition of Coagulants to Activated Sludge

Iron and aluminum salts can be added to activated sludge for phosphorus removal without hindering the biological functions of the bacteria [85,86]. In one study [87], it was found that a weight ratio of ferric to phosphate ions of 1.5 to 1.0 resulted in 83 percent reduction in phosphorus. This ratio would require a ferric chloride dose of about 85 mg/l at the Palo Alto plant. Table 26 shows the results of an experiment in which ferric chloride and C-31 were added to activated sludge. About 30 percent of the SON was removed with a dose of 200-300 mg/l of ferric chloride (although a significantly, 99% CI, larger percentage could be removed by a separate coagulation process), and the effluent was quite clear. An added advantage of direct addition to activated sludge is that separate coagulation and sedimentation tanks are not required, resulting in a much less expensive operation.

The effectiveness of polyelectrolytes when used directly with activated sludge, rather than with secondary effluent, was also tested. The cationic polymer C-31, was able to produce a small but significant (99% CI) reduction in SON and SCOD (16 and 21 percent, respectively) when added directly to activated sludge at a concentration of 20 mg/l, while the addition of A-23, the anionic polymer, increased COD and turbidity and had no noticeable effect on the rate of settling. Busch and Stumm [84] found the polymeric material excreted by bacteria to be much like an anionic polymer. The fact that only the cationic polymer was able to improve settling and organics removal in this study also indicate the anionic nature of the soluble organics present in the activated sludge. The data indicate that the practice of adding a cationic polymer to activated sludge for improved settling may also result in slightly increased removal of soluble organic matter; however, no experiments were undertaken to determine the steady-state effect of such practice, with the sludge being recycled to the aeration tanks and re-coagulated continuously.

Summary

With respect to secondary effluent:

1. Ferric chloride, lime, and alum removed an average of 42 ± 5, 34 ± 5, and 27 ± 3 percent of the SON, respectively, from a number of PASE samples, and an average of 32 ± 6, 24 ± 8, and 28 ± 10 percent of the SCOD, respectively. The percentage differences between coagulants were statistically significant for removal of SON, but not for removal of SCOD. Ferric chloride and lime removed significantly more SON than SCOD.

TABLE 26. REMOVAL OF SON AND SCOD BY ADDITION OF FERRIC CHLORIDE AND C-31 COAGULANT TO ACTIVATED SLUDGE

	$FeCl_3$, mg/1	C-31 mg/1	pH	Turbidity NTU	SON, mg/1	Percent SON Removed	SCOD, mg/1	Percent SCOD Removed
Sample 1†	-	-	7.2	2.6	1.38	-	23	-
	25	-	6.9	1.6	1.68	0	28	0
Organic Nitrogen = 2.2 mg/1	50	-	6.7	1.4	1.61	0	26	0
COD = 32 mg/1	100	-	6.4	0.9	1.17	15	24	0
	300	-	6.2	0.5	0.97	30	18	22
(12-5-75, 11:30 AM)	600	-	6.2	0.7	1.15	17	22	6
SON (> 6%, > 8%)**	900	-	6.2	0.3	1.11	20	19	8
SCOD (> 4%, > 6%)**	600*	-	6.0	0.4	0.86	38	16	29
	-	-	7.2	-	1.09	-	-	-
	200	-	5.8	-	0.78	28	-	-
	300	-	5.9	-	0.79	28	-	-
	400	-	6.0	-	0.82	25	-	-
Sample 2†	600	-	6.0	-	0.90	17	-	-
(12-11-75, 11:00 AM)	200	5	5.7	-	0.70	36	-	-
SON (> 7%, > 10%)**	300	5	5.8	-	0.75	31	-	-
	400	5	5.9	-	0.81	26	-	-
	600	5	5.9	-	0.82	25	-	-
	600*	-	5.8	-	0.57	48	-	-

†Settling time = 45 minutes.

*Added to supernatant siphoned from settled activated sludge.

**Differences in percent removal to be exceeded for 95% and 99% significance, respectively.

2. Sequential coagulation of samples, using different coagulants or pH values, indicated differences in the fractions of organic matter removed by the various coagulants and the dependence of removal of certain fractions upon pH and surface charge.

3. Significant additional removals of SON were achieved with ferric chloride or lime after alum coagulation, ferric chloride at pH 6.0 after ferric chloride at pH 10.0, ferric chloride at pH 10.0 after ferric chloride at pH 6.0, and lime after ferric chloride at pH 6.0 or lime.

4. Optimum coagulant doses of ferric chloride, lime, and alum were about 200-300 mg/l for maximum removal of SON from PASE.

5. Polyelectrolytes created rapidly settling floc, but were of no benefit in removing SON.

6. Bentonite improved SON removal by about 10 percent at a dose of 200 mg/l when added prior to ferric chloride coagulation, but no increased removal was found with kaolinite.

With respect to direct addition to activated sludge:

1. Small reductions in SON and SCOD, 16 ± 3 and 21 ± 2 percent, respectively, were attained by direct addition of cationic polymer to a sample of activated sludge.

2. Direct addition of ferric chloride to activated sludge resulted in about 30 percent removal of SON at a dose of 200 mg/l. Although a higher removal of SON could be attained by separate coagulation of the supernatant liquor, direct addition merits consideration for economic reasons.

ION EXCHANGE AND ACTIVATED-CARBON ADSORPTION

Background

Ion exchange and activated-carbon adsorption are discussed together here due to the similarity of the mechanisms by which they remove organic contaminants. For the purposes of this study, ion exchange is considered to be an adsorption process in which the dominant factor affecting adsorption is electrostatic charge.

Removal of organic contaminants from wastewater by adsorption is influenced by three factors: (1) electrostatic charge, (2) polarity, and (3) special factors. In the case of ion-exchange resins, adsorption is ideally dependent solely on electrostatic charge; however, in the adsorption of organic molecules by the resins, there also can be interactions between the molecules and the resin matrix. This is particularly true of non-ionic organic molecules. For ionized organic molecules, adsorption will be the result of the superimposed effects of electrostatic charge, polarity, and special factors.

There are a large number of anionic and cationic ion-exchange resins commercially available which differ in acid/base strength, pore size, functional group, matrix characteristics, mesh size, ion selectivity, and other parameters. It is not within the scope of this study to determine which of these resins can achieve optimum removal of SON nor to determine how the various characteristics of the resins affect the removal of SON. Rather, a resin was selected which was hoped would favor electrostatic charge over other factors affecting adsorption.

Cleaver and Cassidy [91], observing the adsorption of amino acids on various resins, postulated that resins of lower equivalent weight would be more polar, thus decreasing the non-polar surface area available for adsorption of the aromatic and aliphatic parts of the molecules. They found Dowex-50 to have the lowest equivalent weight of the resins investigated. In this study, a resin equivalent to Dowex-50, Bio-Rad AG50W-X8, was used together with its anionic counterpart, Bio-Rad AG1-X8.

Adsorption of organics from secondary effluent on ion-exchange resins has largely been used as an analytical tool due to the more advanced state of the art of activated-carbon adsorption, although resins are being developed with the hope of equaling, complementing, or surpassing the performance of activated carbon.

Rebhun and Kaufman [92] investigated the removal of COD and color from secondary effluent and found that the weak base phenolic resins, Duolite A-7 and ES-33, achieved the greatest removal of the resins tested, and were roughly comparable to activated carbon. A non-ionic phenolic resin was also found to achieve significant removal of COD and color, thus indicating the importance of polarity and matrix characteristics for adsorption of organics from secondary effluent. Little or no removals of COD and color were observed with strong cationic resins, further supporting the postulation that secondary effluent organics are negatively charged at neutral pH.

Parkin and McCarty [73] studied the removal of SON from a sample of treated agricultural wastewater and found that an anionic resin achieved greater SON removal than a cationic resin, and that either raising or lowering the pH from the neutral range improved removal.

Of particular interest to this study is the work of several investigators who have used ion exchange chromatography to separate amino acids [102,103, 104,105,106], nucleic acid degradation products [107], and ribonucleotides [108]. These investigators used cation exchange resins and were able to adsorb these nitrogen-containing organics at low pH, and elute them from exchange columns with buffers of increasing pH. For example, Moore and Stein [103] adsorbed 17 amino acids onto a column of Dowex-50, and eluted 8 of them with a buffer of pH 3.4, and 6 more with a buffer of pH 4.2.

Activated carbon has been shown to have a high affinity for adsorbing organic molecules from solution, and granular activated carbon has been used at several treatment plants for polishing secondary effluent, well known plants being located at Lake Tahoe [126], Dallas [21], and Pomona [127]. The technology for designing and operating granular activated-carbon adsorption units has been developed and tested and is ready for immediate use [111].

There are a number of different carbon sources and techniques used in the manufacture of activated carbons, producing carbons with a wide range of pore sizes, specific surface areas, acidities, and other parameters which may affect their performance. Various investigators have discussed the catalytic and adsorbent properties of activated carbon [119], its surface chemistry [120,121], and the kinetics, equilibrium, and capacity for adsorption on activated carbon [122,123]. Activated carbon is known to possess ion exchange properties [119], and has been shown by Urano [124] to adsorb heavy metals from solution.

Activated carbon may be either acidic or basic, depending primarily on the temperature and method of its regeneration [119], which may affect its performance with a particular solute. The pH at which adsorption takes place is also important, as a decrease in pH has been shown to increase adsorption capacity [123], and an acidic activated-carbon bed in sequence after a basic bed has been shown to accomplish an additional removal of organics from surface waters [116,125]. However, the concentration of material which cannot be adsorbed from secondary effluent by activated carbon is reportedly not significantly affected by pH changes [73,128,129].

Kim et al. [128] have observed that the cell residence time (CRT) of the activated sludge is an important variable in activated-carbon adsorption of secondary effluent organics, the non-adsorbable fraction decreasing with increasing CRT, but the adsorbable fraction remaining relatively constant. Earlier studies by DeWalle and Chian [28,130] indicated that low-molecular-weight polar compounds constituted a significant portion of the non-adsorbable organics, and that these compounds tended to be removed by aerobic biological treatment, suggesting agreement with and prediction of the results of Kim et al.

Parkin and McCarty [73] contacted a sample of biologically treated agricultural wastewater with 50 g/1 of activated carbon and observed an SON removal of 94 percent.

Helfgott et al. [13] observed a significant positively charged organic fraction in activated-carbon effluent, indicated both by cation exchange and by use of gravitational electrophoresis. The resin used was in the sodium form, so presumably the adsorption took place at neutral pH.

Experimental Procedure

The ion-exchange resins used were analytical-grade purchased from Bio-Rad Laboratories (Richmond, California). The manufacturers' specifications are shown in Table 27. The resins were rinsed with dilute acid or base, then rinsed with sodium chloride for conversion to the sodium and chloride forms, and then rinsed with a large quantity of deionized water. The deionized water was drawn from the resins by vacuum filtration through a fritted glass filter, and the resins were then stored for use. The water content of the resins was

TABLE 27. MANUFACTURERS' SPECIFICATIONS FOR THE ION-EXCHANGE RESINS USED

Type:	Cation	Anion
Name:	AG 50W-X8	AG1-X8
Form:	Hydrogen	Chloride
Total Capacity (meq/dry g):	5.1	3.2
Actual Wet Mesh Range (U.S. Standard):	40-80	40-80
Moisture Content (weight %):	50-56	39-45
Functional Group:	Sulfonic Acid	Quaternary Ammonium
Effective Pore Size:	Medium	Medium

determined to be 47 percent for the cationic resin and 44 percent for the anionic resin by drying at 103°C for several hours.

In batch studies 15 g/l of resin were added to Pall-filtered samples of PASE in erlenmeyer flasks, and then shaken on a "wristaction" shaker (Burrell Corp., Pittsburgh, Pa.) for 12 hours. As shown in Table 28 this dosage was sufficient to remove virtually all of the exchangeable (adsorbable) SON. Resins were removed from samples by filtration through glass fiber filters (Reeve Angel 934 AH), except for samples contacted at pH 9 or higher, which were settled and then decanted, since filtration here was hindered by formation of a precipitate.

Hydrochloric acid and sodium hydroxide were used for pH adjustment, with the exception that sulfuric acid was used where noted to prevent chloride interference in the SCOD test. The pH was checked often if necessary and adjusted to maintain a constant pH in the appropriate experiments.

Levels of SON and SCOD in "blank" deionized water samples contacted with the resins and with activated carbon were found to be satisfactorily low, as shown in Table 29. These low-level SON values were measured by a variation of the technique described in Standard Methods [3], in which 350 ml of the sample was first distilled to ensure complete removal of the ammonia, followed by digestion as usual. Then only 250 ml of deionized water was added following digestion, and approximately 125 ml was distilled into 25 ml of 0.02 N sulfuric acid, with the final volume brought to 200 ml in a volumetric flask. This method extended the sensitivity of the analysis to a lower level by producing a final volume of only 200 ml, as compared with 500 ml in the standard procedure. Using this technique, recoveries of standards were accurate within ± 5 percent down to a level of 50 micrograms of organic nitrogen per flask (0.10 mg/l for a 500-ml sample). Below this level results were erratic, and usually high. Thus, all of the SON values shown in Table 29 are below the detection limit for the analysis.

Grab PASE samples were Pall filtered immediately upon return to the laboratory, and were then either used at once or stored at 4°C until warmed to

TABLE 28. EFFECT OF SEQUENTIAL CONTACTING AND DOSAGE OF ION-EXCHANGE RESINS ON REMOVALS FROM PASE

	First Exchange	Dose, g/1	Second Exchange	Dose, g/1	SON, mg/1	Percent SON Removal[c]	SCOD, mg/1	Percent SCOD Removal[d]	Final pH
Sample 1	-	-	-	-	1.27	-	23	-	7.7[a]
	Cationic	15	-	-	1.16	9	22	5	8.0[a]
	Cationic	15	Cationic	15	1.14	10	21	9	8.2[a]
	Anionic	15	-	-	1.12	12	15	34	7.5[a]
	Anionic	15	Anionic	15	1.13	11	13	41	7.5[a]
Sample 2	-	0	-	-	1.27	-	23	-	2.0[b]
	Cationic	5	-	-	0.88	31	18	21	2.0[b]
	Cationic	15	-	-	0.68	47	19	18	2.0[b]
	Cationic	25	-	-	0.68	47	19	17	2.0[b]
	Cationic	50	-	-	0.64	50	20	14	2.0[b]

[a] pH not adjusted.

[b] pH adjusted with H_2SO_4.

[c] Differences in percent SON removal greater than 6% and 9% are 95% and 99% significant, respectively.

[d] Differences in percent SCOD removal greater than 4% and 6% are 95% and 99% significant, respectively.

TABLE 29. SON AND SCOD OF DEIONIZED WATER SAMPLES CONTACTED WITH ION-EXCHANGE RESINS AND ACTIVATED CARBON

Solid	Initial pH	Final pH	SON, mg/l	SCOD, mg/l
Anionic	2.0	2.0	0.04	8.9*
Anionic	7.6	4.4	0.02	0.0
Anionic	12.0	11.9	0.05	4.5*
Cationic	2.0	2.2	0.03	5.5*
Cationic	7.6	7.4	0.01	1.6
Cationic	12.0	12.0	0.04	5.1*
Cationic	3.0	4.3	0.01	1.6
Cationic	4.0	5.9	0.07	1.2
Cationic	5.0	6.8	0.04	0.4
Cationic	10.0	10.2	0.00	1.4
Cationic	11.0	11.1	0.00	1.6
Anionic	3.0	3.1	0.01	0.6
Anionic	4.0	4.1	0.05	0.0
Anionic	5.0	4.3	0.04	0.0
Anionic	10.0	5.6	0.03	0.0
Anionic	11.0	10.7	0.05	0.8
Cationic	2.0	2.3	-	0.4**
Cationic	12.0	12.1	-	1.2**
Anionic	12.0	12.0	-	1.2**
Activated Carbon	7.0	-	0.01	0.0
Activated Carbon	7.0	-	0.05	0.0
Activated Carbon	12.0	12.0	0.00	0.0**
Activated Carbon	2.0	2.2	0.00	0.0**

*Chloride interference in SCOD test.

**pH adjusted with sulfuric acid in place of hydrochloric acid.

room temperature for use. All experiments were conducted at room temperature (approximately 20°C).

The activated carbon, Nuchar WV-G, 12 x 40 mesh, was washed with deionized water in an upflow column to remove carbon fines, and then dried at 103°C and stored in an airtight container until used. In the batch activated-carbon experiments 25 g/l of carbon (except in the case of the pH-dependence experiment) were added to Pall-filtered PASE in an erlenmeyer flask, which was then shaken for 12 hours on the "wristaction" shaker. Equilibrium was reached after 14 hours with a 10 g/l dose, thus the selection of a 12-hour contact time for a 25 g/l dose appears sufficient for maximum adsorption. The carbon was removed from the samples by filtration through glass fiber filters (Reeve Angel 934 AH), which were found to produce identical results to filtration through 0.45 micron Millipore filters.

Results and Discussion

The effect of pH on removal of SON and SCOD by ion-exchange resins is shown in Figs. 5 and 6, respectively. Below pH 5, adsorption of SON and SCOD by the cationic resin increased sharply, while removal by the anionic resin was relatively independent of pH. In Fig. 5, differences in percent SON removal of 5% and 7% are 95% and 99% significant, respectively; and in Fig. 6, differences in percent SCOD removal of 3% and 5% are 95% and 99% significant, respectively.

The data for adsorption by both resins were obtained from samples which were contacted with a mixture of the two resins (mixed resins). When removal by mixed resins equals the sum of the removal accomplished by the resins individually, as for SCOD above pH 5, then mutually exclusive fractions of the organics have been removed by the individual resins. The SON data at pH 2 and 3, on the other hand, indicate that at least some portion of the same fraction was removed by both resins. Thus at least part of the removal by the cationic resin at low pH must be due either to adsorption of largely neutral compounds or to adsorption of compounds having both positive and negative charges.

It appears that as the pH is lowered, some of the negatively charged organics become neutral, allowing them to adsorb to either resin, while some of the neutral or negatively charged molecules become positively charged, allowing them to be removed only by the cationic resin. In the case of the anionic resin, some molecules become neutral and adsorb, while others become positive and do not adsorb, thus keeping the percent removal by the anionic resins relatively constant. The SON removed at high pH appears to be partly amphoteric; however, some removal may also be caused by precipitation due to the high pH.

In order to determine which fractions were mutually exclusive and which were not, a series of samples were contacted sequentially with both resins at various pH values. The results presented in Table 30 indicate that cationic and anionic resins remove mutually exclusive fractions of SON and SCOD at neutral pH (Sample 1), and that the SON and SCOD removed by anionic resin at both high and low pH contain at least some different material (Sample 2). The fraction of SON and SCOD removed by cation exchange at low pH are significantly (99% CI) distinct from those removed by anion exchange at high pH (Sample 3). Changes in the sequence of contacting caused no significant changes in the results, nor did contacting with both resins simultaneously.

It has been postulated that a significant fraction of the organic matter which is not adsorbable on activated carbon is composed of small polar compounds [28]. Ion exchange should favor the removal of compounds which are small (since they have a higher charge density if charged and easier access to the pores), and apparently favors the removal of compounds which can be favorably protonated or deprotonated (which would most likely also be quite polar since only polar functional groups can be readily protonated or

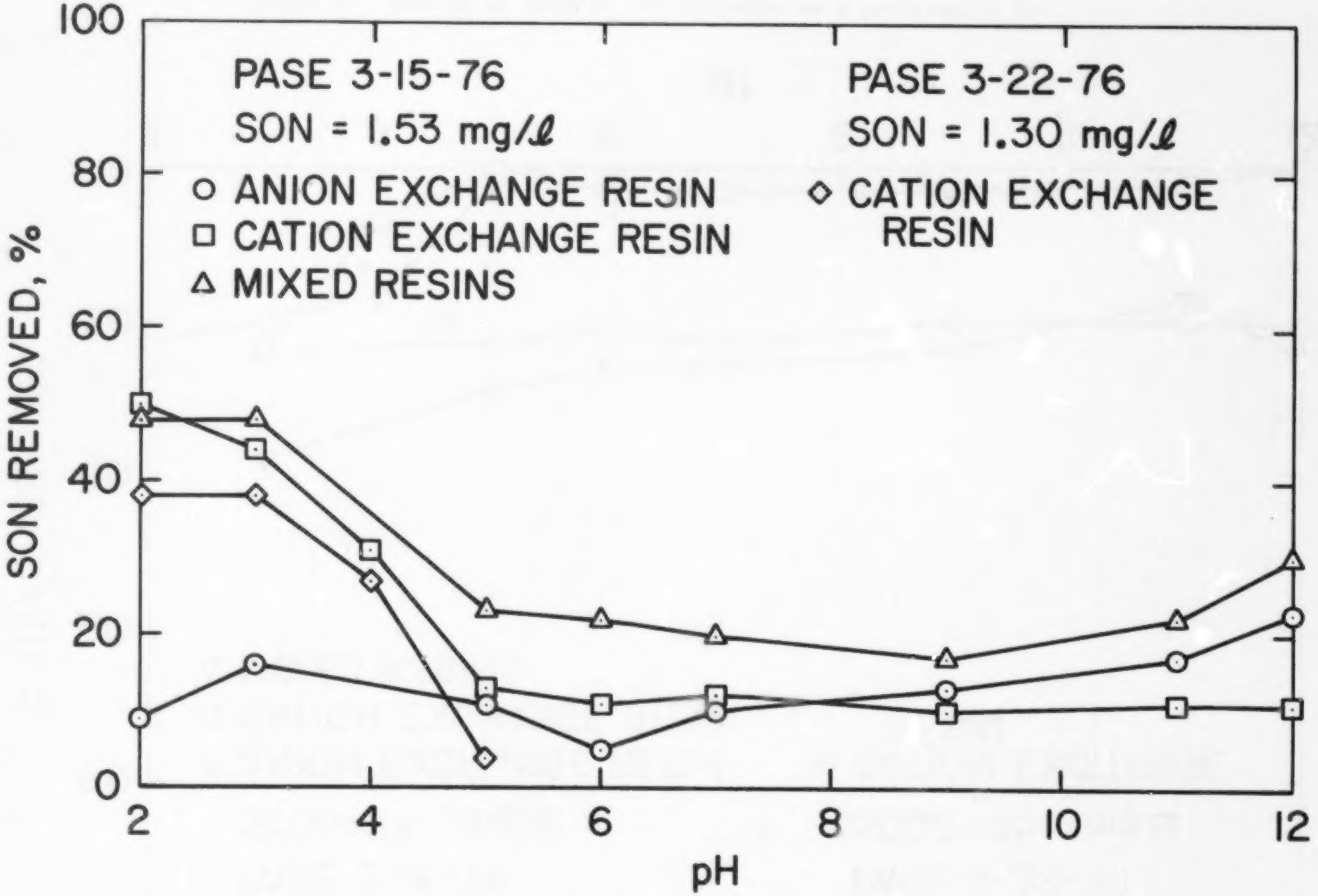


Figure 5. Removal of SON by ion exchange as a function of pH.

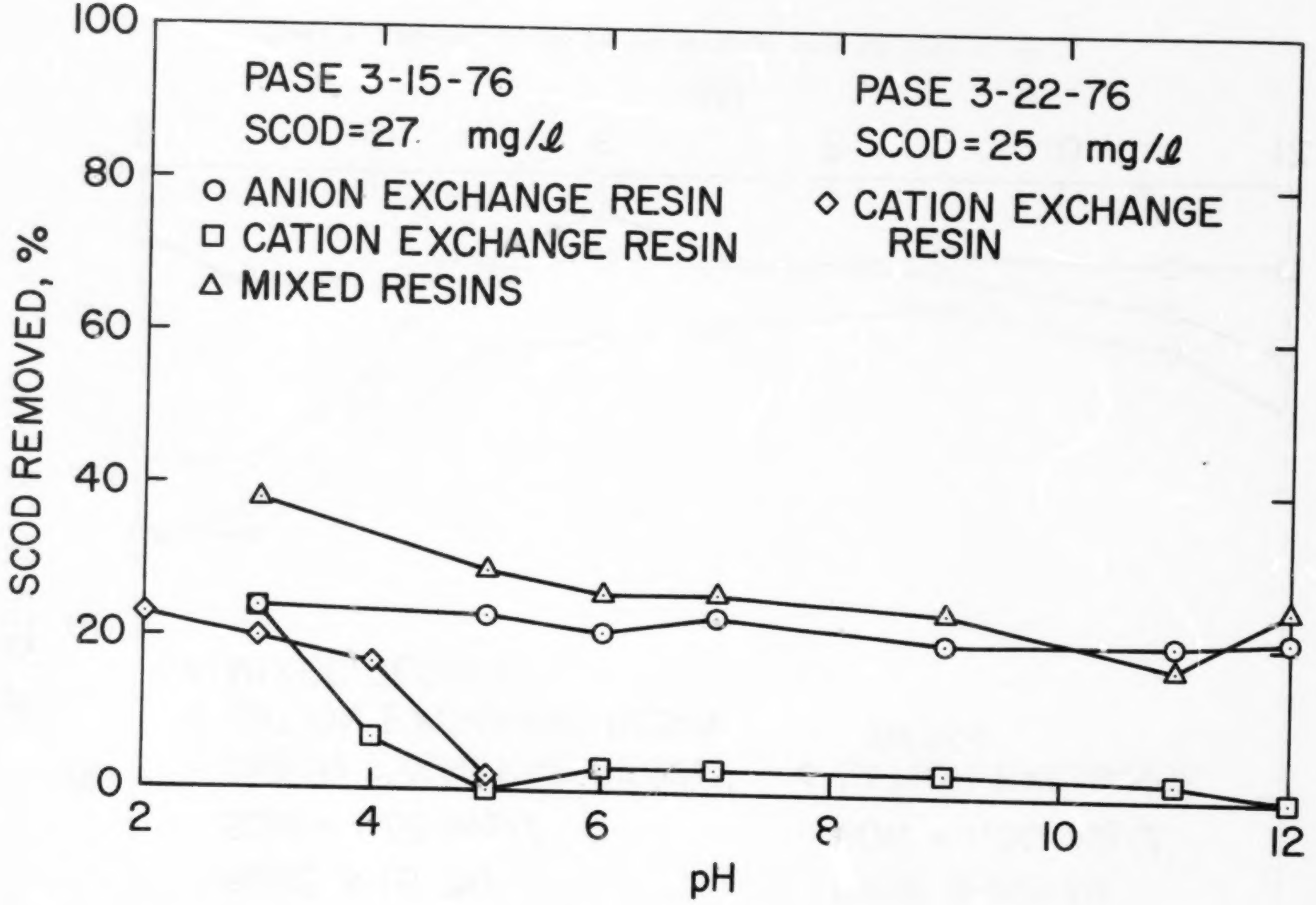


Figure 6. Removal of SCOD by ion exchange as a function of pH.

TABLE 30. REMOVAL OF SON AND SCOD BY SEQUENTIAL CONTACTING WITH ION-EXCHANGE RESINS

	First Resin	First pH	Second Resin	Second pH	SON, mg/l	Percent SON Removed	SCOD, mg/l	Percent SCOD Removed
Sample 1	-	7.8	-	-	1.70	-	29	-
(2-6-76)	Cationic	8.0	-	-	1.50	12	30	0
SON(5%,6%)[b]	Anionic	7.5	-	-	1.53	10	20	32
SCOD(3%,4%)[b]	Anionic	7.5	Cationic	7.8	1.23	28	18	37
	Cationic	8.0	Anionic	7.6	1.25	26	20	33
	Mixed Resins	7.4	-	-	1.25	26	19	33
Sample 2	-	7.6	-	-	1.32	-	24	-
(3-1-76)	Cationic	11.0	-	-	1.23	7	24	0
SON(6%,8%)[b]	Cationic	7.9	-	-	1.16	12	21	12
SCOD(4%,5%)[b]	Cationic	3.2	-	-	0.74	44	18	26
	Cationic	3.2	Cationic	11.0	0.73	45	19	20
	Cationic	11.0	Cationic	3.3	0.79	40	19	20
	Anionic	2.8	-	-	0.96	27	16	34
	Anionic	7.4	-	-	1.06	20	16	32
	Anionic	10.5	-	-	1.02	23	17	29
	Anionic	2.8	Anionic	10.6	0.75	43	13	45
	Anionic	10.5	Anionic	3.1	0.87	34	15	37
Sample 3	-	7.7	-	-	1.30	-	25	-
(3-22-76)	Cationic	2.0[a]	-	-	0.81	38	19	23
	Anionic	12.0[a]	-	-	1.21	7	19	24
SON(6%,8%)[b]	Anionic	12.0[a]	Cationic	2.0[a]	0.65	50	13	48
SCOD(4%,5%)[b]	Cationic	2.0[a]	Anionic	12.0[a]	0.68	47	-	-

[a] pH adjusted with sulfuric acid and sodium hydroxide.

[b] Differences in removal to be exceeded for 95% and 99% significance, respectively.

deprotonated as a function of pH). Thus one might expect to find differences in the fractions of organic matter removed by ion-exchange and activated-carbon adsorption.

The data of previous investigators support this hypothesis [13,129], as do the data presented in Tables 30 and 31. Cation exchange by itself removed 12% of the SON which about equals the 14% SON removal by cation exchange following activated-carbon adsorption (95% CI = 6%). This indicates activated carbon and cation exchange remove mutually exclusive fractions of SON. The high SON to SCOD ratio of this fraction indicates its small molecular weight, since it contains a significant amount of positively charged nitrogen and almost no SCOD. Small amino acids could account for such results if they were present, but as discussed earlier, this is unlikely. Nucleic acid bases would also exhibit similar characteristics, and these may in fact be present.

TABLE 31. REMOVAL OF SON AND SCOD BY ACTIVATED CARBON AND ION-EXCHANGE RESINS

First Contacting	pH	Second Contacting	pH	SON, mg/l	Percent SON Removed[a]	SCOD, mg/l	Percent SCOD Removed[b]
Control	7.7	-	-	1.70	-	29	-
Activated Carbon	8.7	-	-	0.81	52	9	68
Activated Carbon	8.7	Anionic resin	7.8	0.72	58	9	70
Activated Carbon	8.7	Cationic resin	8.9	0.57	66	9	70
Activated Carbon	8.7	Mixed resins	7.8	0.54	68	8	73
Cationic resin	8.0	-	-	1.50	12	30	0
Anionic resin	7.5	-	-	1.53	10	20	32
Cationic resin	8.0	Anionic resin	7.6	1.25	26	20	33

[a] Differences in percent SON removal greater than 5% and 6% are 95% and 99% significant, respectively.

[b] Differences in percent SCOD removal greater than 3% and 4% are 95% and 99% significant, respectively.

As shown in Tables 31 and 32, the SON removed by anion exchange is only partially removed by activated carbon, but the SCOD removed by anion exchange is almost completely removed by activated carbon. There may, however, be anionic molecules present in secondary effluent which are not removable with the anion-exchange resin used in this study.

The data of Parkin and McCarty [73] indicate that the pH at which adsorption occurs does not affect the removal of SON by activated carbon. In this study also, the equilibrium concentration of SON in samples of PASE contacted with 25 g/l of carbon was found to be independent of pH (Table 33). The effect of increasing pH on activated-carbon adsorption is described by Weber [123] as increasing the adsorptive capacity of the carbon, which would not be observable at such a large dose of carbon as 25 g/l, since the capacity of the carbon would greatly exceed the amount of material to be removed. When samples of PASE were contacted with only 400 mg/l of carbon, the effect of decreasing pH in increasing the adsorptive capacity of the carbon for SON was readily apparent, but the effect was not large.

It appears that the carbon used had a maximum adsorptive capacity for SON and SCOD at pH 3. Since the magnitude of the fraction of non-adsorpable organics was not a function of pH, it may be concluded that electrostatic charge is not an important factor in the removal of organics by activated carbon. It can be concluded that polarity is a very important factor in activated-carbon adsorption, since (1) polarity and electrostatic charge are the two principal characteristics of the organics removed by ion exchange; (2) electrostatic charge is not important in activated-carbon adsorption; and (3) compounds removed by cation exchange seem to be poorly adsorbed by activated carbon.

TABLE 32. REMOVAL OF SON AND SCOD FROM TREATED PASE AT NEUTRAL pH

Sample Treatment Prior to Ion Exchange	Additional SON Removed by Cation Exchange, mg/l	Additional SCOD Removed by Cation Exchange, mg/l	Additional SON Removed by Anion Exchange, mg/l	Additional SCOD Removed by Anion Exchange, mg/l
Sample #1				
Control	0.20	0.0	0.17	9
Activated Carbon Adsorption (neutral pH)	0.24	0.0	0.09	1
Sample #2*				
Control			0.31	12
Activated Carbon Adsorption (neutral pH)			0.11	1

TABLE 33. REMOVAL OF SON AND SCOD BY ACTIVATED CARBON AS A FUNCTION OF pH

	Contacting pH^a	SON, mg/l	Percent SON Removed	SCOD, mg/l	Percent SCOD Removed
25 g/l Activated Carbon	Control	1.45	-	26	-
	2.0	0.39	73	4	84
SON (6%, 8%)^b	3.0	0.39	73	4	84
SCOD (4%, 5%)^b	4.0	0.39	73	6	79
	7.0	0.38	74	6	78
	12.0	0.39	73	6	76
400 mg/l Activated Carbon					
Sample 1	Control	1.32	-	25	-
	2.0	0.90	32	13	49
SON (6%, 8%)^b	3.0	0.78	41	15	38
SCOD (4%, 5%)^b	5.0	0.97	26	16	34
	7.8	0.97	26	18	29
Sample 2	Control	1.52	-	24	-
	2.0	1.14	25	14	45
SON (5%, 7%)^b	3.0	1.09	28	13	46
SCOD (4%, 5%)^b	5.0	1.18	22	16	32
	7.0	1.21	20	17	29

[a] pH adjusted with sulfuric acid and sodium hydroxide.

[b] Differences in removal to be exceeded for 95% and 99% significance, respectively.

Table 34 presents a summary of the activated-carbon adsorption data. Activated carbon removed 71 ± 12 percent of the SON and 81 ± 8 percent of the SCOD from PASE. Removal of SON was significantly (95% CI) less than removal of SCOD.

TABLE 34. SUMMARY OF ACTIVATED-CARBON ADSORPTION DATA AT NEUTRAL pH

Activated Carbon, g/l	Initial SON Concentration, mg/l	Percent SON Removed	Initial SCOD Concentration, mg/l	Percent SCOD Removed
25	1.70	52	29	68
25	1.45	74	26	78
25	1.59	75	28	89
Column	0.89	70	18	85
25	1.18	92	22	93
10	0.71	76	21	73
15	1.32	71	27	84
15	1.00	56	24	79
		Avg. 71 ± 12 (n=8)		Avg. 81 ± 8 (n=8)

Table 35 presents a summary of the SON and SCOD removals achieved by ion exchange. Cation exchange removed significantly more SON than SCOD at neutral pH (95% CI) and at pH 2 (99% CI). Anion exchange removed more SCOD than SON (99% CI). Both resins removed about the same amount of SON at neutral pH.

Summary

1. The PASE samples contacted in this study with activated carbon exhibited an average SON removal of 71 ± 12 percent, and an average SCOD removal of 81 ± 8 percent. Activated carbon removed significantly more SCOD than SON.

2. The adsorptive capacity of Nuchar WV-G for adsorbable SON increased with decreasing pH to pH 3.0, but the magnitude of the non-adsorbable SON fraction was independent of pH.

3. Cation exchange at neutral pH removed 11 ± 1 percent of the SON and 4 ± 5 percent of the SCOD from samples of PASE. Anion exchange at neutral pH removed 12 ± 4 percent of the SON and 30 ± 4 percent of the SCOD from samples of PASE. Cation exchange removed significantly more SON than SCOD, while anion exchange removed significantly more SCOD than SON at neutral pH.

4. With a cation-exchange resin, decreasing pH sharply increased the removal of both SON and SCOD, the percentage removed being significantly greater for SON than SCOD. At pH 2.0 the cationic resin

TABLE 35. SUMMARY OF SON AND SCOD REMOVAL BY ION EXCHANGE

Resin*	pH	Initial SON Concentration, mg/l	Percent SON Removed	Initial SCOD Concentration, mg/l	Percent SCOD Removed
Cationic	8.0	1.27	9	23	5
Cationic	7.0	1.53	12	27	3
Cationic	8.0	1.70	12	29	0
Cationic	7.9	1.32	12	24	12
Cationic	8.0	1.25	12	22	2
Cationic	8.0	1.21	11	22	0
			Avg. 11 ± 1		Avg. 4 ± 5
Anionic	7.5	1.27	12	23	34
Anionic	7.0	1.53	10	27	23
Anionic	7.5	1.70	10	29	32
Anionic	7.4	1.32	20	24	32
Anionic	7.6	1.25	9	22	33
Anionic	8.0	1.21	9	22	27
			Avg. 12 ± 4		Avg. 30 ± 4
Cationic	2.0	1.27	47	23	18
Cationic	2.0	1.30	38	25	23
Cationic	3.2	1.32	44	24	26
Cationic	2.0	1.59	39	28	24
Cationic	2.0	1.25	32	22	18
Cationic	2.0	1.53	50	-	-
			Avg. 42 ± 7		Avg. 22 ± 4

*15 g/l of resin used for all samples.

removed 42 ± 7 percent of the SON and 22 ± 4 percent of the SCOD from samples of PASE.

5. Sequential contacting of PASE with ion-exchange resins revealed that the fractions of SON and SCOD removed by the cationic resin at pH 2 and by the anionic resin at pH 12 were significantly different (99% CI), as were the fractions of SON and SCOD removed by the individual resins at neutral pH. However, there appeared to be some overlapping of the fractions removed by the two resins at both high pH and low pH.

6. Contacting of activated-carbon effluent with ion-exchange resins at neutral pH revealed that the cation exchangeable SON was not significantly adsorbed by activated carbon, a portion of the anion exchangeable SON was adsorbed, and the anion exchangeable SCOD was almost completely adsorbed.

CHEMICAL OXIDATION

Background

The chemical oxidants relevant to this study are those generally capable of oxidizing organic compounds in dilute aqueous solution and those particularly capable of oxidizing soluble nitrogen-containing organics. There are two ways in which SON can be effectively removed by oxidation: (1) deamination, in which carbon-nitrogen bonds are broken, and the nitrogen measurable by the Kjeldahl procedure is changed to inorganic nitrogen, and (2) N-oxidation, in which the nitrogen is raised to a higher oxidation state not detected by the Kjeldahl analysis for organic nitrogen. It is not within the scope of this study to identify the end products of oxidation, either quantitatively or qualitatively, but it is quite possible that in some cases they may be more undesirable environmentally than the compounds from which they are formed.

The chemical oxidants evaluated were sodium hypochlorite, ozone, potassium permanganate, and hydrogen peroxide, all of which are capable of oxidizing certain organic functional groups in aqueous solution. Chlorine and ozone have been used extensively for the disinfection of drinking water for many years, as well as for taste, odor, and color removal, generally involving dosages in the milligram-per-liter range.

Chlorine has been the primary disinfectant in the United States, and is added variously as the hypochlorite salt, as chlorine gas, or as chlorine dioxide, depending upon availability and economics. It has long been recognized that nitrogenous organic compounds interfere in this chlorination process, due to their slow reaction with chlorine [131]. Whereas some compounds, such as ammonia, present a rapidly satisfied chlorine demand, organic nitrogen imposes a slowly satisfied demand which requires a combination of an excess of chlorine and an extended contact time to ensure a stable chlorine residual.

In recent years, chlorine has been increasingly applied in the treatment of wastewater for control of noxious odors, to remove color, or to decrease coliform counts. In the tertiary treatment process of breakpoint chlorination, chlorine is used to remove ammonia from secondary effluent. This generally requires a chlorine to ammonia nitrogen ratio of about 10 to 1, compared to a theoretical ratio of 7.5 to 1. The difference is attributed to the presence of organic and inorganic impurities which exert a chlorine demand, and for this reason more highly treated wastewaters generally exert a decreased chlorine demand.

The effect of breakpoint chlorination on organic matter has received relatively little attention, primarily because its primary goal is ammonia removal. It is generally found to accomplish a small reduction in COD, but there is disagreement as to its effect on the organic nitrogen present in secondary effluent. Lawrence et al. [134] found a significant decrease in organic nitrogen below the breakpoint ratio, and complete removal at the breakpoint. Parkin and McCarty [73] found increasing SON removal with increasing chlorine concentration far in excess of the breakpoint, with 42 percent removal in the presence of 130 mg/l free residual chlorine. In both studies, the samples were dechlorinated by bubbling with nitrogen gas at pH 2, a procedure which is not likely to dechlorinate organo-chloro compounds. Pressley et al. [135] found insignificant removal of organic nitrogen by breakpoint chlorination.

A number of investigators have studied the effects of chlorination on specific compounds, many of which may be present in some form in secondary effluent. Wright [132,133] studied the reaction of hypochlorite with amino acids and proteins and concluded that (1) hypochlorite can act either as an oxidizing or as a chlorinating agent, (2) acidity favors chlorination and alkalinity favors oxidation, and (3) the chloramino derivatives formed by chlorination are of variable stability.

Murphy et al. [136] and Sung [137] studied the chlorination of a number of organic compounds thought to be representative of those present in secondary effluent. Among the compounds Murphy and his co-workers found to be easily chlorinated were amines, pyrrole, phenols, amino groups, aldehydes, and ketones, while Sung found that amino acids, uric acid, tannic acid, and humic acid interfered in the disinfection of wastewater by chlorine.

Taras [138,139] studied the effect of chlorine on a number of specific nitrogenous compounds, mostly amino acids, peptides, and proteins, and measured the decrease in albumoid [3] and total nitrogen. Using a chlorine dose of between 0.5 and 1.0 g/l, he found reductions in total nitrogen of about 50 percent for many amino acids, and 10-20 percent for proteins after a 24-hour contact time. A contact time of one hour exhibited slightly smaller removals of amino acid nitrogen, but almost negligible removal of protein nitrogen.

Morris [141] classified the reactions of hypochlorite with organics in dilute aqueous solution into four categories: (1) addition to olefinic bonds; (2) activated ionic substitution, exemplified by the chlorination of phenol and the haloform reaction; (3) oxidation, with reduction of hypochlorite to chloride; and (4) substitution of chlorine for hydrogen on a nitrogen atom. He stated the chlorine which reacts to substitute on nitrogen does not lose its oxidizing capacity; this is supported by the finding of Sung [137] that a number of organochloro derivatives titrate as chlorine residual.

Morris [141] reported that reaction of chlorine occurs with amines, amides, amino acids, proteins, heterocyclic compounds, and often proceeds rapidly, especially with the more basic nitrogen atoms. Alpha amino acids first give N-chloro derivatives, but then, in general, oxidative deamination occurs yielding a keto acid and ammonia chloroamine. Of the heterocyclic compounds, pyrimidine reacts readily, forming chloroamines and C-chlorinated derivatives, purine appears to be simultaneously oxidized and chlorinated, and pyrroles and indoles seem to undergo aromatic substitution; however, none of these reactions has been investigated at the concentrations and conditions of water chlorination.

Ozone has been widely used in Europe for many years to disinfect water supplied. It is one of the strongest oxidants known, considerably stronger than chlorine, and leaves no residual ions or taste in the water. Ozone decomposes rapidly to oxygen in water, especially at high pH [142]. In a recent review of the chemistry of ozone in water, Peleg [143] concluded that the dissociation products of ozone in water may be more powerful oxidizing agents than ozone itself, and that the hydroxyl radical is mainly responsible for the high oxidative potential of ozone in water.

In recent years, a number of investigators have studied the effect of ozone on raw, treated, and industrial wastewaters. Roan et al. [144] ozonated raw wastewater, secondary effluent, lime-clarified and filtered raw and secondary wastewaters, carbon-treated wastewater, and chlorinated and carbon-treated wastewaters. In all effluents except raw wastewater, 100 mg/l of ozone produced at least 70 percent removal of COD. Variable amounts of organic nitrogen and ammonia were oxidized at pH 7, and organic and ammonia oxidation increased with increased pretreatment and with increased pH.

Nebel et al. [145] treated a municipal/industrial secondary effluent with 15 mg/l of ozone in a froth flotation process and found removals of 29 percent of COD, 23 percent of SCOD, 79 percent of color, 70 percent of turbidity, 78 percent of phosphate, 12 percent of nitrate, and suspended solids down to 0-2 mg/l. TOC reduction was not significant and BOD reduction averaged 15 percent, but sometimes the BOD increased during treatment, indicating that ozone can oxidize refractory compounds to biodegradable ones.

Kirk et al. [146] studied the ozonation of secondary effluents and concluded that almost all of the COD could ultimately be oxidized by ozone, but that after 50-70 percent reduction, the reaction rates become extremely extended. The pH of the effluent following ozone treatment was found to always be nearer neutrality than the influent pH, and increased COD removal was found with increased initial pH for a one-hour contact time. Oxidation of ammonia was found to occur only at high pH.

Singer and Zilli [147] investigated the oxidation of ammonia with ozone, and found the reaction to be first order with respect to ammonia concentration, the rate increasing with increasing pH. Ozonation of secondary effluent resulted in about 30 percent removal of COD, but insignificant ammonia removal at neutral pH. Raising and buffering the pH of the effluent at 9.0 resulted in rapid oxidation of the ammonia, but no reduction in COD.

Bailey has written several reviews concerning the reactions of ozone with organic compounds [154,155,156]. He reported that various organic substances with a nucleophilic atom in their structure were readily oxidized by ozone: tertiary anines to amine oxides, and primary and secondary amines to nitro compounds, nitroxides, and various decomposition products. The oxidation of certain amines proceeded faster than that of olefinic double bonds. The majority of ozone-related research has involved solvents other than water and temperatures other than 20°C. In aqueous media, it appears that the major reaction of ozone with amines is side-chain oxidation rather than nitroxide formation, and in acidic solutions amines are unreactive toward ozone due to the unavailability of the nitrogen electron pair. Various amino acids and proteins have been ozonized in water solution, but the ozone attack appears to occur at sulphur or aromatic or heterocyclic unsaturated carbon-carbon bonds rather than nitrogen. The carbon-nitrogen double bond of many compounds is readily cleaved by ozone.

The ozonation of amines has been investigated by a number of researchers [158,159,160,161,162,163,164,165]. Hewes and Davison [160] ozonated a water containing diisopropylamine at pH 7 and found 80-85 percent removal of the amine and 50 percent removal of the amine COD, and suggested that ozone attacked the amino group preferentially.

Other nitrogen-containing compounds and functional groups have also been examined for their reactivity toward ozone. Erickson et al. [166] found that carbon-nitrogen double bonds were as reactive toward ozone as carbon-carbon bonds if they were properly substituted. Wibaut [167] examined the mechanisms of ozone attack on pyrroles. Mudd et al. [168] found the order of susceptibility of amino acids in aqueous solution to ozone attack to be cysteine, methionine, tryptophano, tyrosine, histidine, cystine, and phenylalanine; the other amino acids were unaffected by ozone. Aryl diamines, used as antiozonants in rubber products, were studied by Delman et al. [169], who found that their protective capacity decreased as the size or number of the N-hydrogen substituents increased.

Mosher [170] found that aromatic double bonds added ozone at about 10 percent of the rate of isolated double bonds, and that some highly branched olefins were oxidized readily, while others reacted at about one tenth the rate of ordinary olefinic double bonds. Thus structural relationships are very important in the reactivity of double bonds toward ozone.

Permanganate and hydrogen peroxide have not received much attention as oxidants in wastewater treatment due to their inferior oxidizing ability in water as compared to chlorine and ozone, but they are known to oxidize organic matter under extreme conditions and to attack certain functional groups readily. Medalia [171] carried out "COD-like" tests on pure compounds, comparing permanganate, hydrogen peroxide catalyzed by iron salts, and dichromate, and found peroxide and permanganate to perform only a fraction of the oxidation achieved with dichromate. Felbeck [172] reported that soil humic acids were at least partially degraded by permanganate and peroxide under extreme conditions.

Although potassium permanganate is widely used for taste and odor control and for iron and manganese removal, there is little information available regarding the effect of permanganate on organics under wastewater treatment conditions. Spicher and Skrinde studied potassium permanganate oxidation of organic compounds [173]. They found that permanganate oxidation of organic refractories is more effective under alkaline than under acidic conditions, and that the inorganic constituents of natural river water had no apparent effect on the reaction. They also found the functional group of an organic compound to be important in the reaction, amines, aldehydes, aromatic alcohols, and keto acids being readily oxidized, while carboxylic acids, ketones, aliphatic alcohols, hydroxy acids, amino acids, and some esters were not readily oxidized. The double bond of alkenes was readily oxidized, aldehydes were oxidized to acids, and the benzene rings of phenol and aniline were apparently broken by oxidation with permanganate.

Parkin and McCarty [73] studied the effect of permanganate and hydrogen peroxide on the SON of treated agricultural wastewaters, and found significant removals of SON; however, the results are questionable since the oxidants were not reduced prior to SON analysis. As found by Spicher and Skrinde [125], permanganate oxidation is temperature dependent, as are most oxidation reactions.

Experimental Procedures

All of the samples used in the chemical oxidation experiments were Pall-filtered PASE, either fresh or stored at 4°C, and all experiments were performed at 20°C. The pH of the samples was adjusted with 1.0 and 6.0 N solutions of both hydrochloric acid and sodium hydroxide.

Chlorine was added to the samples as sodium hypochlorite. Chlorine, hydrogen peroxide, and potassium permanganate were generally added to the samples from 10 g/l working solutions, although chlorine was sometimes added from a 5.9 percent stock solution. After a contact time of one hour these oxidants were neutralized with a quantity of 100 g/l sodium sulfite solution sufficient to reduce 1.33 times the initial quantity of oxidant introduced into the sample, leaving an excess of sodium sulfite in the samples. The pH was then adjusted to 7.5-8.0. When reducing residual permanganate, sufficient acid and sulfite were added to allow reduction of the permanganate entirely to the magnanous ion.

The excess sodium sulfite was not removed before the Kjeldahl nitrogen analysis, since it was found that it produced no changes in SON analysis at concentrations as high as 400 mg/l. The removal of excess sulfite ion prior to SCOD analysis was accomplished by adding 10 mg/l of cobaltous chloride, followed by 15 minutes of vigorous aeration with cotton/fiberglass-filtered air introduced through a porous stone diffuser.

One set of chlorinated samples was dechlorinated by the method of Lawrence et al. [134] for comparison. After the one-hour contact time, the pH of these samples was adjusted to 2.0 and they were then aerated for 2 hours with nitrogen gas.

Ozone was generated with a Welsbach Laboratory Ozonator (Model T-408), using pure oxygen feed gas at 0.5 l/min (STP) and 5.5 x 10^6 pa (8.0 psi). Ozone was generated with 100 volts of electricity and carried in the oxygen gas streams through Tygon tubing to the bottom of a plexiglass column where it was released through a glass diffuser into the sample. The plexiglass column was 50 mm in diameter, 1300 mm in overall length, and filled to a depth of approximately 1020 mm with 2.0 liters of sample. The rubber stoppers on the top and bottom of the column were covered with transparent tape (Scotch) to prevent oxidation of the rubber.

The gas escaping from the sample was carried through Tygon tubing and released into a potassium iodide trap through a glass diffuser. The trap was made of a glass tube 29 mm in diameter, 520 mm in length, and was filled to a depth of 380 mm with approximately 250 ml of a 2-percent potassium iodide solution.

Ozone dose was controlled by adjusting ozonation time at constant gas flow rate. For calibration purposes, the flow of gas was short-circuited directly to the potassium iodide trap. The output of the ozonator was checked frequently during use, and was quite constant on any given day. For the first ozone experiment, calibration checks showed that the ozonator produced an average of 579 ± 4 mg of ozone during four separate 20-minute periods.

Samples contacted with ozone were not treated with sodium sulfite after it was determined that the ozone residual was very short-lived and that the addition of sodium sulfite, as described above, had no effect on the results of the SON and SCOD analyses.

Results and Discussion

The results of an experiment in which the removal of SON by several oxidants was studied as a function of applied dose and pH are presented in Table 36. Hypochlorite produced no significant removal of SON and only minimal, if any, removal of SCOD at concentrations from 10-100 mg/l, well below the breakpoint.* Permanganate removed 28 percent of the SON at an optimum pH of 10 and with a dose of 100 mg/l. Since permanganate removed 26 percent of the SON at a dose of 50 mg/l, it does not appear that higher doses will be effective in removing significantly more SON. Peroxide removed 19 percent of the SON at an optimum pH of 11 with a dose of 100 mg/l, although doses of more than 20 mg/l did not remove significantly more SON. Neither peroxide nor permanganate removed a significant (99% CI) amount of SCOD.

These results indicate that some nitrogenous functional groups, most likely the amino groups, are readily and irreversibly oxidized by permanganate and peroxide, and that the effect is greater with permanganate than with peroxide. Spicher and Skrinde [173] found that permanganate reacted readily with amino groups, but not with amino acids, resulting in deamination to form ammonia and organic acids. It is likely that this is also the major reaction of permanganate and peroxide with SON.

If permanganate or peroxide oxidize SCOD, the reaction is probably limited to olefinic double bonds, which are probably too few in number to be reflected in the SCOD. The presence of a high concentration of ammonia nitrogen interferes with the action of hypochlorite, and if organic nitrogen is chlorinated, the reaction is reversed by the addition of sodium sulfite.

Since others [73,134] have reported removal of SON at chlorine concentrations below the breakpoint, using the method of Lawrence et al. [134] for dechlorination, this method was investigated with the results shown in Table 37. At chlorine doses below the breakpoint, it was found that 27 percent of the SON could be reversibly chlorinated, which is, interestingly, about the same percentage of SON oxidized by permanganate. Past the breakpoint, hypochlorite oxidation of the SON appears to be irreversible, as shown by the fact that both methods of dechlorination produced identical results.

---

*The breakpoint is the chlorine dosage required for complete oxidation of ammonia, which requires a chlorine to ammonia ratio of about 8 for PASE [175].

TABLE 36. REMOVAL OF SON AND SCOD BY CHLORINE, PERMANGANATE, AND PEROXIDE

Oxidant	Dose,[a] mg/l	pH	SON, mg/l	Percent SON Removed[b]	SCOD, mg/l	Percent SCOD Removed[c]
(Control)	-	7.4	1.19	-	25	-
Hypochlorite	10	7.0	1.18	1	25	0
Hypochlorite	20	7.0	1.19	0	25	0
Hypochlorite	50	7.0	1.17	2	24	3
Hypochlorite	100	7.0	1.18	1	24	6
Permanganate	10	10.0	1.03	14	-	-
Permanganate	20	10.0	0.93	22	-	-
Permanganate	50	10.0	0.88	26	-	-
Permanganate	100	10.0	0.86	28	-	-
Permanganate	100	9.0	1.02	14	25	2
Permanganate	100	10.0	0.86	28	26	0
Permanganate	100	11.0	0.92	23	24	5
Permanganate	100	12.0	0.94	21	25	2
Peroxide	10	11.0	1.06	11	24	3
Peroxide	20	11.0	0.98	17	25	0
Peroxide	50	11.0	0.97	18	26	0
Peroxide	100	11.0	0.96	19	25	2
Peroxide	100	9.0	0.98	17	25	2
Peroxide	100	7.0	1.10	7	25	2
Peroxide	100	3.0	1.05	12	25	0

[a] As chlorine, potassium permanganate, and hydrogen peroxide.

[b] Differences in SON removal greater than 7% and 9% are 95% and 99% significant, respectively.

[c] Differences in SCOD removal greater than 4% and 5% are 95% and 99% significant, respectively.

The removal of SON by breakpoint chlorination, as a function of dose and pH, is shown in Table 38, and indicates that just beyond the breakpoint (Cl/N = 10.4), all of the chlorine-oxidizable SCOD has been oxidized, but that some of the SON requires a higher dose to be irreversibly oxidized. A pH of 7 appears to be optimum for removal of organics by breakpoint chlorination, but removal is not very dependent upon pH.

Table 39 results indicate the effect of ozone dose and pH on SON removal. An increase in pH from 7.0 to 10.0 resulted in a significant (99% CI) increase in SON and SCOD removal, and a large increase in ammonia removal. The increases in SON and SCOD removal with increasing pH were associated with the increase in absorbed ozone. An undetermined optimum appears to lie at some pH below 12. Removal of SON was slight (95% CI) at doses below 100 mg/l, and SCOD removal increased with increasing ozone dose. The SON removal achieved

TABLE 37. EFFECT OF DECHLORINATION METHOD OF LAWRENCE ET AL. [134]

	Chlorine dose, mg/l	SON, mg/l	Percent SON Removed
Sample 1*	0	1.27	-
	10	1.17	8
	20	1.05	17
	50	0.93	27
	100	0.93	27
Sample 2	0	1.51	-
	472	0.81	46
	472	0.83**	45**

*Same sample as in Table 36.

**Dechlorinated with sodium sulfite.

by ozonation of Sample 1, Table 39, was uncharacteristically high, as can be seen by comparison of the removals of SON and SCOD by high dosages of chlorine and ozone with several samples, as shown in Table 40.

On the average, chlorine achieved 42 percent SON and 18 percent SCOD removals, almost the reverse of that achieved by ozone of 14 percent SON and 46 percent SCOD. It seemed likely from these results that the two were removing different fractions of the organic matter, or attacking different functional groups. The results of two experiments in which chlorine and ozone were used in sequence (Table 41) shows that when ozone is preceded by chlorine, the total removal of SON and SCOD is equal to the sum of the removals accomplished by each oxidant individually. However, when ozonation precedes chlorination, SON removal is greatly increased, while SCOD removal is not increased additionally by the chlorination.

Thus, it appears that the major reaction in the ozonation of SON is side-chain oxidation, which "frees" the nitrogen to a form which can easily be chlorinated, perhaps as amino nitrogen. Additional SCOD is not removed by the chlorine, since ozone is a stronger oxidant and has already oxidized all of the easily oxidizable organics, with the exception of a large part of the readily chlorinated SON, which appears immune to attack by ozone.

Since cobalt ion catalyzes the reaction of sulfite ion with oxygen, it was considered possible that it could act as a catalyst for ozone, but its only effect was to increase the rate of decomposition of the ozone, with no effect on SON or SCOD removal. Bicarbonate ion was found by Denisov et al.

TABLE 38. REMOVAL OF SON AND SCOD BY BREAKPOINT CHLORINATION

	Chlorine Dose, mg/1	Residual Chlorine mg/1	Chlorine: Nitrogen Ratio	pH	SON, mg/1	Percent SON Removed	SCOD, mg/1	Percent SCOD Removed	NH_3-N mg/1	NO_3-N_1 mg/1	NO_2-N mg/1
Sample 1	0	-	-	-	1.55	-	28	-	22.7	0.2[a]	-
SON (5%,7%)[b]	118	83	5.2	7.0	1.55	0	27	3	17.8	0.4[a]	-
	236	34	10.4	7.0	1.13	27	23	18	0.7	1.1[a]	-
SCOD (3%,5%)[b]	354	99	15.6	7.0	0.94	39	23	18	1.4	1.7[a]	-
	472	245	20.8	7.0	0.94	39	23	18	1.1	1.8[a]	-
Sample 2	0	-	-	7.8	1.45	-	26	-	21.0	0.0	0.02
SON (6%,8%)[b]	472	245	22.6	3.0	1.02	30	23	11	4.7	0.8	0.00
	472	177	22.6	7.0	0.89	39	21	18	1.2	1.8	0.00
SCOD (4%,5%)[b]	472	284	22.6	10.0	0.95	34	21	18	0.3	0.2	0.01
	472	333	22.6	12.0	-	-	23	11	3.2	0.3	0.43

[a] Includes nitrite nitrogen.

[b] Differences in removal to be exceeded for 95% and 99% significance, respectively.

TABLE 39. REMOVAL OF SON AND SCOD BY OZONATION

	Initial pH	Final pH	Ozone Applied, mg/1	Ozone Absorbed, mg/1	SON, mg/1	Percent SON Removed	SCOD, mg/1	Percent SCOD Removed	NH_3-N_1 mg/1	Percent NH_3-N Removed	NO_3-N_1 [a] mg/1
Sample 1	7.7	-	-	-	1.76	-	34	-	27.2	-	0.6
SON (5%,6%)[b]	7.7	7.7	289	114	1.26	28	19	45	23.7	13	3.7
	3.0	3.3	362	86	1.34	24	25	25	25.8	5	1.5
SCOD (3%,4%)[b]	10.0	9.1	289	193	1.10	37	17	50	14.8	46	12.3
	12.0	12.3	289	229	1.07	39	18	46	20.3	25	6.5
Sample 2	7.4	-	-	-	1.54	-	28	-	22.9	-	0.4
SON (5%,7%)[b]	7.4	7.4	14.9	13.7	1.43	7	24	14	22.9	0	0.4
	7.4	7.3	74.6	50.0	1.44	7	19	33	21.8	5	1.3
SCOD (3%,5%)[b]	7.4	7.3	149	79	1.45	6	18	36	21.7	5	2.1
	7.4	7.2	299	112	1.32	14	16	44	19.7	14	3.4

[a] Includes nitrite nitrogen.

[b] Differences in removal to be exceeded for 95% and 99% significance, respectively.

TABLE 40. COMPARISON OF SON AND SCOD REMOVALS BY CHLORINE AND OZONE

Oxidant	Dose, mg/l	Initial SON, mg/l	Final SON, mg/l	Percent SON Removed	Initial SCOD, mg/l	Final SCOD, mg/l	Percent SCOD Removed
Chlorine	472	1.51	0.83	45	-	-	-
Chlorine	472	1.55	0.94	39	28	23	18
Chlorine	472	1.45	0.89	39	26	21	18
Chlorine*	472	1.54	0.80	48	24	18	28
Chlorine**	472	1.54	0.91	41	29	26	10
				(Avg.) 42			(Avg.) 18
Ozone	114	1.76	1.26	28	34	19	45
Ozone	112	1.54	1.32	14	28	16	44
Ozone	44	1.16	1.03	11	24	11	53
Ozone	104	1.08	1.02	6	25	14	45
Ozone	115	1.25	1.14	9	22	11	51
Ozone*	108	1.54	1.26	18	24	13	45
Ozone**	106	1.54	1.38	11	29	17	41
				(Avg.) 14			(Avg.) 46

*,** indicate same sample.

[174] to inhibit the oxidation of alcohols by hydrogen peroxide, but it was found to have no effect on ozonation other than to buffer the pH of the wastewater during ozonation.

Summary

1. When added to a sample of PASE at pH 7.0, chlorine dosages less than 100 mg/l (below the breakpoint) produced no significant removal of SON and very little (6 ± 2%) removal of SCOD.

2. When added to a sample of PASE adjusted to various values of pH, potassium permanganate and hydrogen peroxide removed a maximum of 28 ± 3 and 19 ± 3 percent of the SON, respectively, at pH 10.0 and 11.0, respectively, and produced no significant removal of SCOD.

3. A chlorine to ammonia nitrogen ratio of approximately 20:1 removed 42 ± 4 percent of the SON and 18 ± 7 percent of the SCOD from PASE. A significantly (99% CI) lower amount of oxidation of SON was found for a 10.4:1 ratio, but SCOD removal was unaffected by an increase above a ratio of about 10.

4. Ozonation removed 14 ± 7 percent of the SON and 46 ± 4 percent of the SCOD from PASE, at absorbed doses mostly in excess of 100 mg/l and at neutral pH.

5. Ozone and chlorine were found to remove 14 ± 7 and 42 ± 4 percent of SON, respectively, and these fractions were found to be mutually exclusive. Removal of SON by chlorine was increased about 30 percent by preozonation.

PROCESSES IN SEQUENCE

Background

Sequences of processes not discussed previously are presented herein. Sequences of one coagulant with another, one ion-exchange resin with another or with activated carbon, or one oxidant with another were discussed previously.

The goals of this portion of the study were: (1) to evaluate the removal of SON by sequences of processes used in wastewater treatment; (2) to distinguish differences and similarities in the fractions of SON removed by some of the different processes; and (3) to identify any synergistic or antagonistic effects arising from the sequential use of processes which would make it desirable to arrange them in a particular order or substitute one process for another. It is not within the scope of this study to attempt to optimize SON removal or to evaluate all possible sequences of treatment processes. Many of the processes are used primarily for other purposes than SON and SCOD removal, and their sequence is generally dictated by other considerations, although great flexibility exists in designing such systems.

Ozone is a relative newcomer to the wastewater treatment field. Many of the experiments described in this chapter dealt with ozone as one of a sequence of treatment processes.

There is little or no information in the literature relating to the removal of SON by sequences of treatment processes. Schertenleib [175] found that overall removal of COD was unaffected by the order of breakpoint chlorination and activated-carbon adsorption in a laboratory study. No evidence was found in the literature for unexpected antagonistic or synergistic effects in the removal of organics by a sequence of advanced waste-treatment processes.

Spicher and Skrinde [125] found that the inorganic constituents of natural river water had no apparent effect on the reaction of potassium permanganate with organic refractories, but there is little or no information available on the effect of the large number and kinds of inorganic ions in secondary effluent on the oxidation of organic contaminants. If inorganic ions do catalyze or inhibit oxidation reactions, the position of ion exchange in a wastewater treatment sequence may be highly important.

To evaluate the removal of SON by sequences of processes currently being used or considered for advanced wastewater treatment, samples were obtained from the South Tahoe Public Utility District Water Reclamation Plant, and other samples were generated in a bench-scale laboratory advanced wastewater-treatment plant, using the design criteria of the planned Palo Alto Water Reclamation Facility [51].

TABLE 41. REMOVAL OF SON AND SCOD BY COMBINATIONS OF CHLORINE AND OZONE

	Treatment[a]	Final pH	Ozone Applied, mg/1	Ozone Consumed, mg/1	Residual Oxidant, as Chlorine	SON, mg/1	Percent SON Removed	SCOD, mg/1	Percent SCOD Removed
Sample 1	Control	7.4	-	-	-	1.54	-	29	-
SON (5%,7%)[b]	Chlorine	7.0	-	-	252	0.91	41	26	10
SCOD (3%,4%)[b]	Chlorine, then Ozone	6.9	311	204	11	0.74	52	14	51
	Ozone	7.2	301	106	-	1.38	11	17	41
	Ozone, then Chlorine	7.0	301	106	264	0.41	74	18	37
Sample 2	Control	7.8	-	-	-	1.54	-	24	-
SON (5%,7%)[b]	Ozone	7.5	277	108	-	1.26	18	13	45
SCOD (4%,5%)[b]	Chlorine	7.0	-	-	206	0.80	48	18	28
	Ozone, then Chlorine	7.0	277	108	241	0.38	75	10	51

[a] Chlorine applied at 472 mg/1 at pH 7 for 1 hour; ozonation begun with an initial pH of 7.5.

[b] Differences in removal to be exceeded for 95% and 99% significance, respectively.

Experimental Procedures

Samples from the Tahoe plant were collected and stored as described in Section 5. The laboratory-scale advanced wastewater treatment consisted of lime clarification (350 mg/l CaO), followed by ammonia stripping (1600 m^3 air per m^3 wastewater, recarbonation (single stage), mixed-media filtration (240 m^3/m^2-day), breakpoint chlorination (8 mg Cl_2/mg NH_3-N), and finally activated-carbon adsorption. The activated-carbon column was 50 mm in diameter, packed to a depth of 0.6 m with Nuchar WV-G 12 x 40 mesh activated carbon, and the flow rate was 240 m^3/m^2-day. A complete description of the laboratory units and their operation is given elsewhere [51]. The samples used in the laboratory reclamation study were unfiltered chlorinated PASE.

Experiments involving ozone in sequence with alum, lime, ferric chloride, activated carbon, and ion exchange were carried out on Pall-filtered PASE, following the procedures previously outlined, except where noted.

The artificial secondary effluent used in evaluating the interference of ozone and ferric chloride in the Kjeldahl test contained 27 mg/l potassium dihydrogen phosphate, 10 mg/l potassium bicarbonate, 122 mg/l magnesium chloride, 162 mg/l calcium chloride, 8.4 mg/l sodium fluoride, 60 mg/l sodium bicarbonate, 128 mg/l sodium sulfate, 181 mg/l sodium chloride, and 54 mg/l ammonium chloride. This artificial effluent contained approximately the concentrations of the major inorganic ions present in PASE, and no organic matter.

Results and Discussion

The results of the sampling at the Tahoe plant are presented in Table 42. The SON value for secondary effluent is an anomaly. The removal of both SON and SCOD was much less than anticipated, and this is attributed to the fact that plant operation was geared for inorganic nutrient removal rather than removal of organics. Lime was added primarily to raise the pH for ammonia stripping (which was not in operation during the sampling for this study), only half of the wastewater was chlorinated, and the activated-carbon beds were used primarily for dechlorination and were not being regenerated sufficiently for optimum removal of organics. Thus, the results indicate that good removal of organics by advanced treatment may not be achieved unless the plant is operated specifically for that purpose.

The results of the laboratory wastewater treatment study, presented in Table 43, show an average organic nitrogen removal of 88 percent and an average COD removal of 92 percent for three chlorinated PASE samples. Although removals may be somewhat lower in the planned full-scale plant, it appears that advanced wastewater treatment using currently available technology can remove a large percentage of the SON and SCOD from secondary effluent. The data also show that only a small percentage of SON is removed by breakpoint chlorination when there is no large excess of chlorine present. Thus, the majority of the SON removal occurs during coagulation and activated-carbon adsorption.

It was also found during the course of the laboratory study that recarbonation (either single-stage or two-stage), varying the flow rate through the

TABLE 42. REMOVAL OF SON AND SCOD BY THE SOUTH TAHOE P.U.D. WATER RECLAMATION PLANT

Sample	Organic-N, mg/1	SON, mg/1	Percent SON Removed*	COD, mg/1	SCOD, mg/1	Percent SCOD Removed*	Soluble TOC, mg/1	BOD_5, mg/1	Soluble BOD_5, mg/1
Plant Influent	9.3	2.9	-	388	123	-	-	186	64
Secondary Effluent	4.2	1.17	-	84	25	-	10	41	2
Lime-Treated Effluent	1.7	1.55	-	32	25	-	11	5	3
Chlorinated Effluent**	1.7	1.39	10	29	24	3	11	-	-
Filtered Effluent	1.4	1.32	15	24	24	4	10	-	-
Activated-Carbon Effluent	1.2	1.07	31	20	20	19	10	1	< 1

*Assuming lime-treated value = 100%. SON removals greater than 5% and 7% and SCOD removal greater than 4% and 5% are 95% and 99% significant, respectively.

**50 percent of flow was breakpoint chlorinated, then recombined with non-chlorinated flow.

TABLE 43. RESULTS OF LABORATORY ADVANCED WASTEWATER TREATMENT

	Parameter	PASE	Effluent from						Percent Removed
			Lime Treatment	Ammonia Stripping	Recarbonation	Filtration	Breakpoint Chlorination	Activated-Carbon Adsorption	
Sample 1 Noon: 9-30-75	Org-N, mg/l	2.32	1.21	1.10	-	1.18	1.06	0.49	79
	COD, mg/l	38	24	24	-	20	18	6	84
	NH_3-N, mg/l	24.9	24.4	13.7	-	13.3	0.1	1.6	94
	pH	7.5	11.5	11.3	8.2	7.6	7.3	8.1	-
Sample 2 Noon: 10-9-75 SON = 1.24 mg/l	Org-N, mg/l	3.12	0.87	0.83	-	0.79	0.63	0.03	99
	COD, mg/l	45	16	17	-	16	13	0	100
	NH_3-N, mg/l	24.2	18.2	9.3	-	12.1	0.0	0.0	100
	pH	7.4	11.5	11.2	7.9	8.1	7.1	9.3	-
Sample 3 Noon: 10-24-75 SON = 1.33 mg/l	Org-N, mg/l	1.95	0.90	0.85	0.93	0.82	0.79	0.27	86
	COD, mg/l	35	20	21	22	20	17	2	93
	NH_3-N, mg/l	22.0	19.3	11.1	12.9	13.3	0.02	1.7	92
	pH	7.7	11.4	11.2	6.9	-	-	-	-

mixed-media filter, and injecting 5 mg/l of ozone before or after lime treatment all had no significant effect on SON or SCOD removal.

Ozonation was studied in sequence with various chemical coagulants, with the results presented in Table 44. Generally, SON removal was significantly (99% CI) greater when ozonation followed coagulation than when it preceded coagulation. The same was generally true for SCOD removal, except when ozonation at neutral pH preceded lime treatment. Ozonation at high pH following lime treatment was highly effective in removing SON and SCOD, but the absorbed dose of ozone was unusually high.

When ferric chloride coagulation was followed by ozonation, there initially appeared to be a synergistic effect on SON removal, since removal exceeded the sum of the removals by the individual processes. However, it was discovered that this effect was due to an interference in the Kjeldahl analysis caused by ozonation of the supernatant liquid from ferric chloride coagulation. This was verified with "artificial" secondary effluent, having an ionic makeup closely resembling PASE and containing no organic matter, as previously described. A sample of this artificial effluent was coagulated with ferric chloride and settled, and then the supernatant liquid was split into two portions. One portion was ozonated and adsorbed 65 mg/l of ozone. A 250-mg/l volume of each portion was then added to 250 ml of PASE and the resultant 500-ml sample was analyzed for SON. Recovery was 99 percent for the PASE sample combined with the coagulated supernatant, but was only 73 percent for the PASE sample combined with the ozonated ferric chloride supernatant. Thus, ozonated ferric chloride supernatant interferes in the Kjeldahl analysis.

It is possible that this interference was caused by the generation of perchlorate ion, catalyzed by the presence of iron or iron oxide. Perchlorate ion was found to strongly interfere in the Kjeldahl analysis; however, it was not determined whether the perchlorate ion could be formed by ozonation of ferric chloride supernatant liquid.

Table 45 shows the results of an experiment in which ozone was used in various sequences with activated carbon and ion-exchange resins. At neutral pH, removal of SON and SCOD was independent of the order of treatment, indicating there are neither inhibiting/catalyzing inorganic ions present nor synergistic/antagonistic effects between the processes. At neutral pH SON removal was approximately additive for sequences of ozonation and adsorption, thus indicating that the non-adsorbing fractions of SON were more readily attacked by ozone. This is understandable if adsorption is biased toward strongly ionized and hydrophobic (non-polar) molecules, while ozone preferentially attacks polar functional groups.

Cation exchange by itself at pH 2 removed more SON (32%) than the sequence of ozonation followed by cation exchange at pH 2 (20%), and ozonation did not remove a significant amount of SON when following cation exchange at pH 2. Thus, it appears that ozonation alters some of the organics which are removable by low pH cation exchange, and that cation exchange adsorbs a portion of the SON oxidizable by ozone.

TABLE 44. REMOVAL OF SON AND SCOD BY OZONE IN SEQUENCE WITH CHEMICAL COAGULATION

Sample	Treatment[a]	pH after First Treatment	pH after Second Treatment	SON, mg/1	Percent SON Removed[e]	SCOD, mg/1	Percent SCOD Removed[e]
1	Control	7.6	-	1.16	-	24	-
	98 mg/1 ozone[b]	7.6	-	1.03	11	11	53
	600 mg/1 alum	6.0	-	0.81	31	16	33
	400 mg/1 lime	11.6	-	0.66	43	16	33
	1) 98 mg/1 ozone[b]; 2) 600 mg/1 alum	7.6	6.0	0.87	25	8	64
	1) 600 mg/1 alum; 2) 93 mg/1 ozone[b]	6.0	7.5	0.62	47	8	66
	1) 98 mg/1 ozone[b]; 2) 400 mg/1 lime	7.6	11.6	0.76	35	9	62
	1) 400 mg/1 lime; 2) 80 mg/1 ozone[b]	11.6	4.7	0.68	41	11	54
2	Control	7.7	-	1.08	-	25	-
	104 mg/1 ozone	7.7	-	1.02	6	14	45
	400 mg/1 lime	11.5	-	0.61	43	16	37
	1) 104 mg/1 ozone; 2) 400 mg/1 lime	7.7	11.5	0.69	36	9	62
	1) 400 mg/1 lime; 2) 202 mg/1 ozone at pH 11.5	11.5	11.3	0.33	69	5	80
3	Control	7.4	-	1.54	-	29	-
	106 mg/1 ozone[c]	7.2	-	1.38	11	17	41
	600 mg/1 ferric chloride	6.0	-	0.99	36	19	34
	1) 106 mg/1 ozone[c]; 2) 600 mg/1 ferric chloride	7.2	6.0	0.88	43	15	47
	1) 600 mg/1 ferric chloride; 2) 106 mg/1 ozone[c]	6.0	7.5	0.59[d]	61[d]	13	55
4	Control	7.8	-	1.54	-	24	-
	108 mg/1 ozone[c]	7.5	-	1.26	18	13	45
	600 mg/1 ferric chloride	6.0	-	0.85	45	14	44
	1) 600 mg/1 ferric chlor.;2) 84 mg/1 ozone[b]	6.0	7.5	0.55[d]	74[d]	8	65

[a] Ozone doses are absorbed doses.

[b] pH adjusted to 7.6 prior to ozonation.

[c] pH adjusted to 7.5 prior to ozonation.

[d] Probable interference in this analysis.

[e] Consult Table A-2, Appendix A for percent removals required for significance.

TABLE 45. REMOVAL OF SON AND SCOD BY OZONE IN SEQUENCE WITH ION EXCHANGE AND ACTIVATED-CARBON ADSORPTION

Treatment[a]	pH after First Treatment	pH after Second Treatment	SON, mg/l	Percent SON Removed[c]	SCOD, mg/l	Percent SCOD Removed[c]
Control	7.5	-	1.25[b]	-	22[b]	-
115 mg/l ozone	7.5	-	1.14[b]	9	11[b]	51
400 mg/l activated carbon	8.0	-	1.14	9	15	33
1) 115 mg/l ozone; 2) 400 mg/l activated carbon	7.5	7.9	0.99	21	11	58
1) 400 mg/l activated carbon; 2) 88 mg/l ozone	8.0	7.4	0.96	23		51
15 g/l anion exchange resin	7.6	-	1.14	9	15	33
1) 115 mg/l ozone; 2) 15 g/l anion exchange resin	7.5	7.6	1.10	12	7	69
1) 15 g/l anion exchange resin; 2) 82 mg/l ozone	7.6	7.3	1.02	18	7	67
15 g/l cation exchange resin	8.0	-	1.10	12	22	2
1) 115 mg/l ozone; 2) 15 g/l cation exchange resin	7.5	7.9	0.93	25	11	51
1) 15 g/l cation exchange resin; 2) 114 mg/l ozone	8.0	7.6	0.94	25	12	47
15 g/l cation exchange resin	2.0	-	0.85	32	18	18
1) 115 mg/l ozone; 2) 15 g/l cation exchange resin	7.5	2.0	1.00	20	8	62
1) 15 g/l cation exchange resin; 2) 118 mg/l ozone	2.0	6.8	0.82	34	10	55

[a] Ozone doses are absorbed doses; initial pH for ozonation was 7.5; applied ozone dose was 272 mg/l.

[b] Average of 4 analyses.

[c] Differences in SON removal greater than 6% and 7%, and differences in SCOD removal greater than 3% and 4% are 95% and 99% significant, respectively. Differences in SON removal greater than 0.07 and 0.09 mg/l and differences in SCOD removal greater than 0.7 and 1.0 mg/l are 95% and 99% significant, respectively, due to 4 observations of the control sample.

The SON most readily adsorbed by a small dosage of activated carbon appeared distinct from that removed by ozonation (Table 45). In order to evaluate this further, the effluent from an activated-carbon column was ozonated. As shown in Table 46, ozone did not significantly oxidize the SON and SCOD remaining after activated-carbon adsorption, and preozonation did not appear to affect removal of SCOD by activated carbon. Thus, SCOD which was attacked by ozone was also removed by activated carbon.

The results of the ferric chloride coagulation experiments, in which coagulation was carried out sequentially at pH 6.0 and 10.0 (see Chemical Coagulation) indicated that there are fractions of the organics which are electrostatically removed, i.e., they behave as anions or cations. Since such fractions might be expected to be closely related to the material removed by the ion-exchange resins, an experiment was conducted to determine if this was indeed the case. As shown in Tables 47 and 48, the fraction removed

TABLE 46. REMOVAL OF SON AND SCOD BY OZONATION AND ACTIVATED-CARBON COLUMN ADSORPTION

Treatment	SON, mg/l	Percent SON Removed[e]	SCOD, mg/l	Percent SCOD Removed[e]
Control[a]	0.89	-	18	-
Activated-carbon adsorption[b]	0.27	70	3	85
47 mg/l ozone[c]	0.94	0	13	28
8 mg/l ozone[c]	0.89	0	17	8
1) 47 mg/l ozone[c]; 2) Activated-carbon adsorption	0.43	52	3	83
1) Activated-carbon adsorption; 2) 41 mg/l ozone	0.41[d]	54	3	86
1) Activated-carbon adsorption; 2) 4.5 mg/l ozone	0.23	74	2	87

[a] Control is lime-treated, air-stripped, recarbonated, and filtered PASE; treatment parameters were those given for the laboratory advanced wastewater treatment.

[b] Carbon column was 50 mm in diameter, packed with 0.6 m of Nuchar WV-G, 12 x 40 mesh; flow rate was 240 m^3/m^2-day.

[c] Ozone doses are absorbed doses.

[d] Perhaps an analytical error; value should be less than 0.27 mg/l.

[e] Differences in SON removal greater than 9% and 12% and differences in SCOD removal greater than 5% and 7% are 95% and 99% significant, respectively.

TABLE 47. REMOVAL OF SON AND SCOD BY FERRIC CHLORIDE COAGULATION AND ION EXCHANGE

Sample	First Treatment[a]	pH after First Treatment	Second Treatment[a]	pH after Second Treatment	SON, mg/1	Percent SON Removed[b]	SCOD. mg/1	Percent SCOD Removed[b]
1 (Control)	-	7.6	-	-	1.21	-	22	-
2	Anion Exchange	8.0	-	-	1.10	9	16	27
3	Cation Exchange	8.0	-	-	1.07	11	23	0
4	Ferric Chloride	6.0	-	-	0.79	35	15	34
5	Ferric Chloride	10.0	-	-	0.83	31	17	24
6	Ferric Chloride	6.0	Anion Exchange	8.0	0.67	45	9	58
7	Ferric Chloride	6.0	Cation Exchange	8.0	0.63	48	14	35
8	Ferric Chloride	10.0	Anion Exchange	8.0	0.73	39	11	49
9	Ferric Chloride	10.0	Cation Exchange	8.0	0.83	31	17	24

[a] Ferric chloride = 600 mg/1 $FeCl_3$ with settling time of 60 minutes; anion and cation exchange with 15 g/1 of resin.

[b] Differences in SON removal greater than 7% and 9% and differences in SCOD removal greater than 4% and 6% are 95% and 99% significant, respectively.

TABLE 48. REMOVAL OF SON AND SCOD FROM COAGULATED PASE BY ION EXCHANGE AT NEUTRAL pH

Sample Treatment Prior to Ion Exchange	Additional SON Removed by Cation Exchange, mg/l	Additional SCOD Removed by Cation Exchange, mg/l	Additional SON Removed by Anion Exchange, mg/l	Additional SCOD Removed by Anion Exchange, mg/l
Control	0.14	0.0	0.11	6.0
Ferric Chloride Coagulation, pH 6.0	0.16	0.2	0.12	5.4
Ferric Chloride Coagulation, pH 10.0	0.00	0.0	0.10	5.6

by the cation-exchange resin was also removed by ferric chloride at pH 10.0. However, the organics removed by anion exchange are different from those removed by ferric chloride coagulation at pH 6.0. This was not anticipated since anionic organic molecules were expected to be removed by both processes. However, anionic molecules which are small and polar are not likely to be adsorbed on the ferric hydroxide surface, but are the preferred molecules for removal by anion-exchange resins. Also, the lattice of the resin is an aromatic and alkyl structure which can adsorb aromatic contaminants under the proper conditions. Thus it is conceivable that the resin adsorbs aromatic and small strongly charged molecules, while ferric chloride coagulation at pH 6.0 removes larger negatively charged molecules.

It was desired to determine whether pretreatment of PASE by any of several processes prior to activated-carbon adsorption would result in lower concentrations of SON and SCOD than when the sample was treated only with activated carbon. As shown in Table 49, none of the various pretreatments resulted in lower SON and SCOD concentrations following activated-carbon adsorption.

The ultraviolet spectra of a number of the samples shown in Table 49 were determined between wavelengths 200 and 320 nm on a Beckman double beam spectrophotometer and absorption was found to decrease with increasing SCOD removal. For Samples 7 and 10, in which SON removal exceeded SCOD removal, the reduction in ultraviolet absorption more closely paralleled SCOD removal than SON removal.

Summary

1. A sequence of advanced wastewater-treatment processes (lime coagulation, ammonia stripping, recarbonation, filtration, breakpoint chlorination, and activated-carbon adsorption) can remove a large amount (perhaps 70-90 percent) of the SON and SCOD from secondary

TABLE 49. REMOVAL OF SON AND SCOD BY COAGULATION, ION EXCHANGE, AND ACTIVATED-CARBON ADSORPTION

Sample	First Treatment	pH	Second Treatment	pH	Third Treatment	pH	SON, mg/1	Percent SON Removed*	SCOD, mg/1	Percent SCOD Removed*
1 (Control)	-	-	-	-	-	-	1.59	-	28	-
2	Fe	10.0	-	-	-	-	1.08	32	20	29
3	Fe	10.0	AC	8.0	-	-	0.38	76	3	89
4	Fe	10.0	AC	8.0	Fe	6.0	0.35	78	4	84
5	Fe	10.0	Fe	6.0	-	-	0.89	44	16	41
6	AC	8.0	-	-	-	-	0.40	75	3	89
7	CAT	8.0	-	-	-	-	1.43	10	28	0
8	CAT	8.0	Al	6.0	-	-	1.04	35	19	31
9	CAT	8.0	Al	6.0	AC	7.9	0.35	78	2	91
10	CAT	2.0	-	-	-	-	0.97	39	21	24

*Differences in SON removal of 6% and 8% and differences in SCOD removal of 4% and 6% are 95% and 99% significant, respectively.

AC = 25 g/l activated carbon
Fe = 600 mg/l ferric chloride
Al = 600 mg/l aluminum sulfate
CAT = 15 g/l cation-exchange resin

effluent, the bulk of the removal occurring during coagulation and activated-carbon adsorption.

2. Removal of SON and SCOD in filtered secondary effluent appears to be more efficient when ozonation follows rather than precedes coagulation. Pretreatment of samples by ion-exchange resins did not result in a significant increase in SON or SCOD removal by ozone.

3. The SON and SCOD remaining after passing the effluent through a column of activated carbon are not readily removed by ozone.

4. The fraction of SON removed by the cation-exchange resin is also removed by ferric chloride coagulation at pH 10, but not at pH 6. The fractions of SON and SCOD removed by the anion-exchange resin are not removed by ferric chloride coagulation at either pH 6 or 10.

5. Pretreatment of PASE with several different processes prior to activated-carbon adsorption resulted in no significant reduction of SON and SCOD compared to the reduction achieved by activated carbon alone.

DISCUSSION OF RESULTS

The physical removal of SON and SCOD by treatment processes and combinations of treatment processes, and the oxidative removal of SON and SCOD is discussed in the following. A scheme was developed to fractionate the SON and SCOD based on the observed removal of different portions by the various processes and combinations of processes. Appendix A contains the statistical basis for discussion of the significance of the results of this portion of the overall study.

Physical Removal Characteristics of SON

Despite the complexity of the systems under consideration and the heterogeneous nature of the organic material present in secondary effluent general conclusions may be drawn with qualification. Realizing the limitations, it is believed that a simplified characterization scheme of the removal of organic contaminants will facilitate this discussion. Such a scheme is developed and employed in the following paragraphs, and is summarized in Table 50. The scheme is useful in explaining the results of the physical removal processes, but not the oxidative processes, the effects of which are related more to molecular structure than to gross molecular characteristics.

Molecular Characteristics Affecting Physical Removal of SON--

The molecules comprising the organic matter in secondary effluent possess a wide range of characteristics, i.e., molecular size, charge, polarity, and other characteristics, such as the presence of a particular functional group or complexing ability, which may affect their removal by the various processes. It is useful to classify organic compounds in terms of these characteristics.

TABLE 50. SUMMARY OF MOLECULAR CHARACTERISTICS AFFECTING REMOVAL OF ORGANIC CONTAMINANTS

		Molecular Characteristics							
		Size		Polarity		Charge			Other*
Process	pH	Small	Large	Polar	Non-polar	Positive	Negative	Neutral	
Chemical Coagulation (Alum or ferric chloride)	6	o	+	-	+	-	+	o	+
Chemical Coagulation (Lime or ferric chloride)	10-12	o	+	-	+	+	-	o	+
Anion Exchange	All	o	-	-	+	-	+	o	+
Cation Exchange	All	o	-	-	+	+	-	o	+
Activated-Carbon Adsorption (Batch)	All	o	-	-	+	-	-	o	+

o Characteristic does not affect removal.
+ Characteristic favors removal.
- Characteristic hinders removal.

*Characteristics which permit the formation of a chemical bond or result in a chemical reaction, accounting for specific adsorption and precipitation.

Regarding size, contaminant molecules may range from molecular weights of less than 100 to small colloids, which in this study are limited to those smaller than 0.45 microns. Molecules are arbitrarily divided into two sizes: small and large. Large molecules include (1) molecules of colloidal size which can be removed by coagulation and flocculation; (2) molecules which can be removed by entrapment during coagulation; and (3) molecules excluded from the pores of activated carbon and ion-exchange resins. Small molecules are those with access to the pores of the ion-exchange resins and activated carbon.

Molecules of increasing polarity tend to be less adsorbable, to be more soluble, and to bind water more tightly. Decreasing polarity favors adsorption to a surface, such as a resin matrix or activated carbon. For simplicity, molecules are classified as either polar or non-polar. Non-polar molecules are those which are hydrophobic, i.e., they would prefer not to be in the water and are thus readily adsorbed on most surfaces. Polar molecules are less readily removed, being hydrophilic.

Molecules may be electrostatically charged, positively or negatively, or they may be neutral. Charged molecules are attracted to surfaces of the opposite charge and repelled by surfaces of like charge.

Molecules may also possess other characteristics which allow them to be removed under otherwise unfavorable conditions. For example, although some carboxylic acid molecules are both negatively charged and polar, they may adsorb to a negatively charged calcium oxide surface because they are capable of forming strong complexes with calcium, or their calcium salts may be insoluble. Similarly, polar molecules may be chemisorbed on activated carbon.

Any one molecule may possess a number of different characteristics, the combination of which will determine its behavior. Distinctive fractions of the organic matter are therefore hard to define and seldom mutually exclusive, leading to qualitative, rather than quantitative, descriptions of results.

Reading across Table 50 for a particular process allows one to determine the dominant characteristics of the fractions of organics removed and not removed by each process. For example, the fraction not removed by activated carbon will contain large, rather polar, and/or charged molecules. A compound possessing only one "hindering" characteristic may not be removed even though it possesses other characteristics favoring removal. Thus, a hydrophobic, positively charged molecule may not be removed by cation exchange if it is too large to penetrate the pores of the resin.

Comparison of SON and SCOD Removals--

Table 51 contains a summary of the removals of SON and SCOD achieved by the various processes. The removals reported were achieved using large dosages to insure maximum removal under batch conditions and to determine the fractions of SON and SCOD removable by each process. Slightly higher or lower removals may be achieved with flow-through system or lower doses, respectively. A more detailed description of each process and the effect of various parameters, including dose and pH, on each process can be found in the appropriate section dealing with that process.

TABLE 51. SUMMARY OF REMOVALS ACHIEVED BY INDIVIDUAL PROCESSES

Process	Number of Samples	pH*	SON Removed, %				SCOD Removed, %			
			Avg.	Max.	Min.	Std. Dev.	Avg.	Max.	Min.	Std. Dev.
Lime Coagulation	9	> 11	33	43	26	6	24	36	13	8
Alum Coagulation	4	6	29	35	23	5	28	46	17	10
Ferric Chloride Coag.	14	6	40	48	30	5	32	40	19	6
Anion Exchange	6	N	12	20	9	4	30	34	23	4
Cation Exchange	6	N	11	12	9	1	4	12	0	5
Cation Exchange	6	2	42	50	32	7	22	26	18	4
Act.-Carbon Adsorp.	8	N	71	92	52	12	81	93	68	8
Chlorination	5	N	42	48	39	4	18	28	10	7
Ozonation	7	N	14	28	6	7	46	53	41	4

*N = neutral.

As shown in Table 51 there are differences between the SON and SCOD removals achieved by several of the various processes. These differences are explainable if consideration is given to the differences one might expect between molecules which contain nitrogen and those which do not. Some of these differences are: (1) only nitrogen-containing molecules are capable of retaining a stable positive charge (with the exception of organo-metallic complexes) since only the nitrogen atom is capable of strongly binding an additional hydrogen atom yielding a positively charged molecule; (2) larger molecules have been reported to be nitrogen enriched [23]; and (3) nitrogen-containing molecules, in general, tend to be more polar than non-nitrogenous molecules, since nitrogen-carbon bonds are more polar than carbon-carbon bonds.

Chemical coagulation with both lime and ferric chloride was found to remove a significantly (95% and 99% CI, respectively) larger percentage of SON than of SCOD. The opposite would be expected from charge and polarity considerations. At pH 12 the calcium oxide surface is negatively charged, as are most organic compounds at that pH, so charge does not favor adsorption. If nitrogen-containing molecules are in fact more polar than molecules which do not contain nitrogen, then polarity would favor the removal of SCOD over SON. Therefore, either nitrogen enrichment of the larger molecules removed by coagulation and/or specific adsorption of nitrogenous molecules must account for the observed difference.

Cation exchange removed significantly (95% CI) more SON than SCOD, while anion exchange removed significantly (99% CI) less SON than SCOD. This indicates the tendency of nitrogenous molecules to be more positively charged. The organic matter removed by the cation-exchange resin appears to be nitrogen enriched, while that removed by the anion-exchange resin is nitrogen deficient. Furthermore, a lowering of pH, which should increase the positive charge of the organics, was found to increase the removal of nitrogen-enriched molecules by cation exchange.

The preference of activated carbon for SCOD adsorption relative to SON may be due to several factors: (1) polarity, since activated carbon removes less polar compounds more readily and nitrogenous compounds are expected to be more polar; (2) molecular size, since the larger molecules (reportedly nitrogen enriched [23]) may be excluded from the pores of the activated carbon; and/or (3) specific adsorption, if the special mechanisms by which activated carbon removes organic molecules favor the removal of non-nitrogenous molecules.

Physical Removal of SON by Combinations of Processes

Factors Affecting Removal--

The molecular characterization scheme in Table 50 is helpful in interpreting the results of the experiments dealing with the physical removal of SON by combinations of processes described in Section 7. The results of these experiments can, in turn, be used to quantify the various fractions of organics described by the scheme.

In the removal of SON by combinations of chemical coagulation processes, different organics fractions were removed by different coagulants or by a particular coagulant at different pH values. Some of these differences suggest that electrostatic attraction is of importance in removal and thus a discussion of surface charge is warranted.

Lengweiler et al. [88] determined that the point of zero charge (PZC) for iron oxides in dilute solutions is at pH 6.7. Aluminum sulfate flocs have a PZC of about 8.0 [54,90]. Various anions in secondary effluent (e.g. sulphate) tend to make the surface of flocs more negative, while various cations (calcium, magnesium) tend to make it more positive [79,80]. Although the ionic makeup of PASE was determined, present knowledge does not permit calculation of a PZC for this system. It is likely that at pH 6.0 ferric chloride and alum flocs in PASE bear a positive charge (although they may conceivably be neutral or very slightly negative) and at pH 10.0 they bear a negative charge. Regardless of the true value of the PZC, both flocs will be more negatively charged at pH 10.0 than at pH 6.0. If charged molecules are present in secondary effluent, those which are negatively charged will adsorb better at low pH, and those which are positively charged will adsorb better at high pH.

There are, of course, other mechanisms, in addition to electrostatic attraction, which are important in the removal of organic contaminants by coagulation, such as co-precipitation, flocculation, and adsorption. In some cases where a second or third coagulation is carried out, these other mechanisms can sometimes be shown to be inoperative. When alum or ferric chloride was used a second time at approximately the same pH, no significant additional removal of SON was achieved (Table 24), nor did largely increased doses of alum and ferric chloride remove significantly more organic material (Tables 20 and 21). Thus, when a second coagulation was performed at a different pH than the first, it can be assumed that any additional removal was not simply due to increased removal by a new surface, by co-precipitation, or by an improvement in overall efficiency.

Removal by Combinations of Coagulants--

The effect of ferric chloride coagulation at two different pH values (6.0 and 10.0) was investigated. When the first coagulation was at pH 6.0, a second coagulation at pH 10.0 resulted in an additional 12 percent removal of SON (Samples 2 and 4, Table 24), reflecting the removal of a significant (99% CI) fraction of SON not removed by the first coagulation. Similarly, when the first coagulation was at pH 10.0 a second coagulation at pH 6.0 resulted in an additional and significant (99% CI) 27 percent removal of SON. The total removal of SON achieved by sequential coagulation with ferric chloride at the two different pH values was the same, regardless of the order of coagulation. An average of about 20 percent of the SON was removable by ferric chloride at both pH 10.0 and 6.0. The removal of this fraction appears to be relatively independent of pH (and therefore surface charge) and is most likely due to a combination of co-precipitation, flocculation, specific adsorption, and entrapment.

That coagulation with ferric chloride at pH 6.0 and pH 10.0 appears to remove different fractions of organic matter can be explained in terms of electrostatic adsorption. If certain fractions of the organic matter are behaving as anions and cations, their removal by ferric chloride will be dependent upon pH. The fraction of organics removed by ferric chloride at pH 10.0 but not at pH 6.0 (comprising about 12 percent of the SON) will be termed "positive"; and the fraction removed at pH 6.0 but not at pH 10.0 (comprising about 27 percent of the SON) will be termed "negative." The terms "positive" and "negative" used here are meant to describe the relative charge of the fractions with respect to each other rather than their absolute charge. Further evidence of the existence and nature of these fractions will be presented later in this discussion.

The addition of lime to secondary effluent (containing carbonate anions) results in the formation of a calcium carbonate/oxide solid, the surface of which is negatively charged at the values of pH used in wastewater treatment [81]. Small amounts of phosphate and other anions present in secondary effluent tend to make the calcium carbonate surface even more negatively charged [79,80,81]. Thus, the surface formed from lime is expected to be negatively charged, as is the surface of the ferric chloride floc at pH 10.0.

Neither lime coagulation following ferric chloride coagulation at pH 10.0 nor ferric chloride coagulation at pH 10.0 following lime coagulation was able to achieve any significant additional removal of SON (Samples 3 and 4, Table 21). Thus, it appears that both coagulants removed the same fractions of organics when the flocs were of the same charge. When lime coagulation followed ferric chloride coagulation at pH 6.0 (flocs likely to be of opposite charge) an average additional removal of SON of 10 percent was achieved (Samples 1 and 4, Table 24), once again indicating a "positive" fraction of organic matter of roughly 10 percent.

When lime coagulation was carried out a second time following an original lime coagulation, an additional and significant 16 percent removal of SON was observed (Sample 1, Table 24). This additional removal may be explained by an expected difference in surface charge for the calcium carbonate surface. In the first coagulation, the surface is more negatively charged because of carbonate, phosphate, and other adsorbing anions, as explained above. These anions are not present for the second coagulation and so the surface is more neutral and for this reason can remove additional organics.

Alum coagulation did not achieve any significant additional removal of SON following ferric chloride coagulation. Thus, there appears to be a fraction of organic matter removable by ferric chloride and not removable by alum coagulation, since ferric chloride was approximately 14 percent more efficient (99% CI) in removing SON than alum (Table 23). This fraction is believed to be specifically adsorbed on the iron hydroxide surface. Interestingly, there was no significant difference detected in the removal of SCOD by alum and ferric chloride but a small difference may have actually occurred. It is possible that the fraction of organic matter removed by ferric chloride and not by alum is nitrogen enriched.

The addition of bentonite prior to ferric chloride coagulation at pH 6.0 increased SON removal by 10 percent (99% CI). While this effect could conceivably be due to an increased efficiency of flocculation, it was not observed with kaolinite clay or any of the polyelectrolytes used in conjunction with either ferric chloride or lime. A more likely explanation for the increase in removal lies in the fact that bentonite is a natural cation exchanger and may have been able to adsorb cationic molecules (the "positive" fraction), which were then removed together with the bentonite upon subsequent ferric chloride coagulation.

Removal by Combinations of Ion Exchange--

The ion-exchange resins evaluated were identical in matrix and cross-linking, differing only in their respective functional groups. Thus, molecules adsorbing on one resin and not the other should do so primarily because they are of the proper charge, even though forces other than electrostatic attraction may be important in the adsorption. For example, Semmens and Gregory [183] found that only the ionized forms of carboxylate ions were adsorbed by anion-exchange resins, even though there were interactions between the carbon chains of the molecules and the resin matrices.

At neutral pH, cation exchange was found to remove 11 ± 1 percent of the SON and 4 ± 5 percent of the SCOD from samples of PASE. Anion exchange was found to remove 12 ± 4 and 30 ± 4 percent of the SON and SCOD, respectively. The fractions of organic matter removed by the two resins at neutral pH were found to be mutually exclusive, and it is very likely that they are also appropriately charged. If so, they represent positively and negatively charged fractions of organic matter.

Removal by Combination of Ion Exchange and Coagulation--

The positively and negatively charged fractions of organic matter defined by ion exchange might be expected to parallel the "positive" and "negative" fractions defined earlier for ferric chloride coagulation. The percentage of SON found to adsorb on the cation-exchange resin agreed well with the percentage of SON termed "positive" as a result of the ferric chloride coagulation experiments. However, the percentage of SON adsorbed on the anion-exchange resin was less than half the percentage predicted to be "negative" by the ferric chloride coagulation experiment.

An experiment was conducted to determine if these fractions were in fact the same. The SON removed by cation exchange was not removed by ferric chloride coagulation at pH 6.0, but it was entirely removed at pH 10.0 (Tables 47 and 48). Thus, the "positive" fraction defined by ion exchange and by ferric chloride coagulation appear to be identical. However, the SON and SCOD adsorbed by the anion-exchange resin were not removed by ferric chloride coagulation at pH of either 6.0 or 10.0. Thus, the "negative" fraction defined by ferric chloride coagulation is separate and distinct from the fraction removed by anion exchange.

It is not clear why the "negative" fraction defined by ferric chloride is not removed by anion exchange. Although the molecules comprising both fractions might be expected to be negatively charged (or neutral), electrostatic charge is not the only important factor affecting adsorption on the two surfaces involved. The ferric hydroxide surface will not adsorb certain negatively charged molecules which are small and polar. The anion-exchange resin used for this study contains quaternary ammonium functional groups, which are probably incapable of true ion exchange for all but the smallest of anionic molecules and are incapable of hydrogen bonding. The lattice of the resin is an aromatic and alkyl structure which may adsorb aromatic contaminants under the proper conditions. Thus, it is conceivable that the resin removes aromatic molecules. A study of the individual molecules removable by each surface should help in resolving this question.

Removal by Combinations Including Activated Carbon--

It has been postulated that a significant fraction of the organic matter which is not adsorbable on activated carbon is composed of small polar compounds [28]. Ion exchange should favor the removal of small charged molecules, since they will have a higher charge density than larger molecules and easier access to the pores of the resin. The charge on a molecule makes it more polar, perhaps preventing adsorption on activated carbon, but may increase the ability of the molecule to adsorb on ion-exchange resins. Thus, one might expect to find differences in the fractions of organic matter removed by ion exchange and activated-carbon adsorption.

Table 31 presents the results of an experiment conducted to compare the fractions removed by ion exchange and activated carbon. These results are summarized in Table 32 in a more readily interpretable form. The organic matter removed by the cation-exchange resin is not adsorbed on activated carbon. Virtually all of the SCOD adsorbed on the anion-exchange resin but only a portion of the SON so adsorbed is removed by activated carbon.

The adsorption of much of the organic matter removable by anion exchange on the activated carbon may reflect the hydrophobic nature of these molecules, and suggests that adsorption on the anion-exchange resin may be largely due to interactions between the molecules and the resin matrix. The anion-exchange resin used may not be able to remove all anions, thus, organic material not removed by activated carbon may be anionic even though not removed by the anion-exchange resin.

Summary of Physical Removal Process Results--

Due to the large number of overlapping mechanisms for removal, the multiple characteristics of the molecules, and the lack of sharp distinction between the divisions of the molecular characteristics, it is difficult to accurately quantify the organics into a complete set of mutually exclusive fractions. However, a few assumptions can be made which will allow the SON to be fractionated into a useful set of categories which can be roughly quantified, as presented in Fig. 7.

The fractionation scheme shown in Fig. 7 is based upon a large number of analyses conducted on a large number of samples. It is thus a composite or summation of the results of this study, and as such is not meant to be a precise fractionation of a single, well-characterized sample. Rather, it is meant to depict the relative sizes of the various fractions and their interrelationships in a simplified and easily understandable form. The size of each fraction has been approximated to within ± 5 percent.

The existence of a small, positively charged fraction of the SON is evidenced by (1) cation exchange; (2) the difference in SON removal with ferric chloride at pH 6.0 and 10.0; (3) the additional 10 percent SON removal achieved by adding bentonite prior to ferric chloride coagulation and (4) the additional 10 percent removal of SON achieved by lime coagulation following ferric chloride coagulation at pH 6.0. This fraction seems to comprise approximately 10 percent of the total SON in all cases. As shown in Table 51, cation exchange at neutral pH removed an average of 11 ± 1 percent of the SON from 6 samples, along with 5 ± 4 percent of the SCOD of the same samples. This fraction is fraction "P" in the fractionation scheme of Fig. 7.

The "negative" fraction, comprising 27 ± 4 percent of the total SON (approximated as 25% in the scheme) was evidenced by the ferric chloride coagulation data as discussed earlier in this section. The "neutral" fraction shown in Fig. 7 is comprised of the organic matter not termed "positive" or "negative." While molecules in the "neutral" fraction may in fact be positively or negatively charged, their charge character is not evident in their behavior in the systems studied. It is quite likely that most of these molecules are negatively charged, but that mechanisms and factors other than electrostatic attraction are dominant in the behavior noted.

The fraction removed by activated carbon comprises 71 ± 12 percent of the SON and 81 ± 8 percent of the SCOD. Since the adsorption of SON by activated carbon is not a function of pH (except for capacity), removal of organics on activated carbon is not related to electrostatic charge, and in fact, charged molecules might be expected to adsorb poorly. As discussed earlier, the positively charged fraction is not removed by activated carbon. The fraction not removed by activated carbon and not positive should consist of molecules which are small, very polar, and possibly negatively charged, assuming that under batch conditions, most large molecules are adsorbed onto the outer surface of the carbon. The fraction of SCOD removed by anion exchange is entirely removed by activated carbon, indicating its hydrophobic nature.

Any given sample of secondary effluent may be fractionated by the scheme shown in Fig. 7 simply by carrying out the treatment processes to define the fractions of interest. This type of fractionation scheme is not limited, however, to secondary effluents or to the treatment processes shown. Any waste

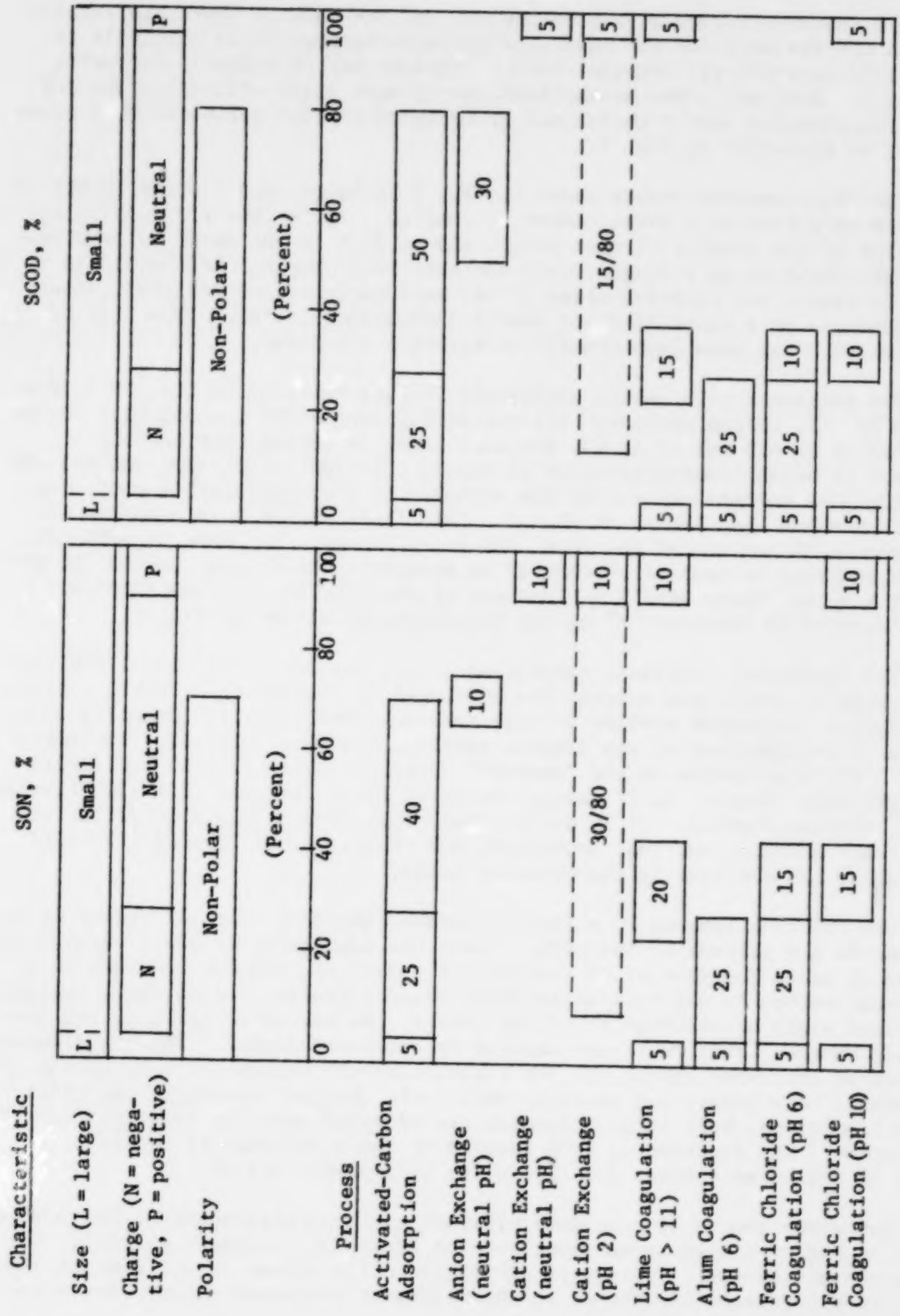

Characteristic
Size (L = large)
Charge (N = negative, P = positive)
Polarity
Process
Activated-Carbon Adsorption
Anion Exchange (neutral pH)
Cation Exchange (neutral pH)
Cation Exchange (pH 2)
Lime Coagulation (pH > 11)
Alum Coagulation (pH 6)
Ferric Chloride Coagulation (pH 6)
Ferric Chloride Coagulation (pH 10)
SON, %
L
Small
N
Neutral
P
Non-Polar
(Percent)
0
20
40
60
80
100
5
25
40
10
10
30/80
10
5
20
10
5
25
5
25
15
5
15
10
SCOD, %
L
Small
N
Neutral
P
Non-Polar
(Percent)
0
20
40
60
80
100
5
25
50
30
5
15/80
5
5
15
5
5
25
5
25
10
5
10
5

Figure 7. Operational fractionation of SON and SCOD.

may be fractionated in a similar manner, provided that care is taken to adequately define the relationships between the various fractions. The important point is that treatment processes do not all remove the same fractions of organic matter and that the differences between the fractions are predictable and understandable from known characteristics of the processes and the molecules being removed.

The fractions shown in Fig. 7 are not all mutually exclusive and a great many assumptions were made in the definitions, descriptions, and in the assigned values. Nevertheless, they are useful in describing the removal of SON from secondary effluent by a sequence of treatment processes and in suggesting directions which future investigations might take. The methods and the principles discussed for secondary effluent may be extended to predict the removal of various fractions for other wastes, perhaps employing other treatment processes.

Oxidative Removal Characteristics of SON

Table 52 contains a summary of the results of the experiments dealing with chemical oxidation of SON.

Potassium permanganate and hydrogen peroxide removed 28 and 19 percent of the SON, respectively, but did not remove any SCOD. Spicher and Skrinde [173] found that permanganate reacts readily with amino groups (but not with amino acids) resulting in deamination to form ammonia and organic acids. This is likely to be the major reaction of permanganate with SON.

Chlorine was found to easily (at low doses) oxidize 27 percent of the SON, but SON thus oxidized could be reduced with sodium sulfite, indicating that the major initial reaction of chlorine with SON is substitution. This again suggests the presence of amino groups, comprising roughly 25-30 percent of the SON.

TABLE 52. SUMMARY OF RESULTS OF CHEMICAL OXIDATION EXPERIMENTS

Oxidant	Dose, mg/1	pH	Percent SON Removed*	Percent SCOD Removed*
Chlorine	452	7	42 ± 4	18 ± 7
Ozone	100	7	14 ± 7	46 ± 4
Potassium Permanganate	100	10	28 ± 3	0 ± 2
Hydrogen Peroxide	100	11	19 ± 3	2 ± 2

*Standard deviations for chlorine and ozone are based on analysis of a number of samples (Table 51); others are analytical standard deviations.

Ozonation resulted in very little SON removal, although a large percentage of the SCOD was removed. Thus SON is quite resistant to ozonation as compared to SCOD; however, the side chains of the nitrogen atoms appear to be altered by ozone, as evidenced by an observed 30 percent increase in SON removal by chlorination after a sample had been ozonated.

In terms of physical removal characteristics, ozone does not appear to attack the "positive" fraction, the fraction removed by anion exchange, nor the SON removed by very small doses of activated carbon. The fraction of the organic matter most strongly oxidized by ozone is the additional matter removed by cation exchange as the pH is lowered from neutral to 2.0. Ozonation is expected to increase the percentage of SON in the "negative" and "polar" fractions and to break up large molecules into smaller ones.

SECTION 8

SON FORMATION AND REMOVAL BY BIOLOGICAL PROCESSES

INTRODUCTION

The research described in this section was conducted to evaluate the source and characteristics of SON in activated-sludge effluents. It would be helpful to know how much SON is produced during treatment, and what factors affect the quantity produced, as well as what fraction of influent SON remains unaltered through treatment. If a large fraction of the effluent SON is produced during biological treatment, the advisability of such treatment may be questioned and ways to minimize the production may be sought. The same could be said for effluent SCOD (soluble chemical oxygen demand). The effect of process control parameters such as mixed liquor suspended solids concentration (MLSS), organic loading, and aeration time on SON production and removal by activated sludge were evaluated in this study to help define operational criteria for minimizing effluent SON.

SON in the effluent from activated-sludge treatment is a function of SON in the influent to the system and biological processes within the system, such as production, utilization, and biodegradation which result in SON charges. Bacteria produce organic nitrogen compounds that are excreted, or released during cell lysis to form SON. Some compounds are biodegradable, some refractory. Bacteria can utilize some organic nitrogen compounds for nitrogen, carbon, and/or for energy. Factors affecting production, utilization, and biodegradability of SON are discussed in the following.

NOMENCLATURE

Equations defining the concentration of various SON types and sources are as follows:

$$SON_i = SON_b + SON_r \quad (1)$$

$$SON_e = SON_r + SON_p + f(SON_b) \quad (2)$$

$$SON_p = SON_g + SON_d \quad (3)$$

$$SON_g = SON_{gb} + SON_{gr} \quad (4)$$

$$SON_d = SON_{db} + SON_{dr} \quad (5)$$

SON_i represents the SON in the influent, and consists of a biodegradable portion, SON_b, and a refractory portion, SON_r.

Activated-sludge effluent SON (SON_e) consists of refractory material originally in the influent and not removed during treatment (SON_r), a fraction of the biodegradable influent SON not removed during treatment ($f(SON_b)$), and SON produced during treatment (SON_p). Theoretically, f can range from 0 - 1 depending on the efficiency of treatment.

SON_p represents material produced as a result of bacterial growth (SON_g) and material produced by organism decay (SON_d). SON_g and SON_d may contain biodegradable SON (SON_{gb} and SON_{db}) and/or refractory SON (SON_{gr} and SON_{dr}).

A major objective of this research was to evaluate the contributions of the SON types listed in Eqs. 1 to 5 to SON contained in activated-sludge effluents. The nature of and factors affecting each form of SON are discussed in the following.

BIOLOGICALLY PRODUCED SON

The major classes of nitrogen-containing organic compounds produced by bacteria are listed in Table 53. Materials present in activated-sludge effluents may be decomposition products, condensation products, or derivatives of these compounds.

From 60 to 80 percent of bacterial cell dry weight is represented by protein and nucleic acids, which contain organic nitrogen [29,30,31,32]. This percentage varies with bacterial species and phase of growth. Nucleic acids (DNA and RNA) and proteins are usually made from amino acids, purines, and pyrimidines that are either synthesized from inorganic nitrogen and simple carbon compounds, or preferably found by some bacteria in the surrounding environment [30]. With the preponderance of organic nitrogen in the cell, it is likely that two processes take place simultaneously during cellular metabolism: (1) utilization of extracellular SON compounds as carbon, energy, and/or nitrogen sources, and (2) excretion of intracellular organic nitrogen metabolites to form biodegradable and refractory SON.

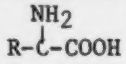
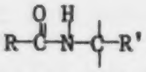
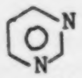
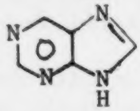
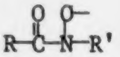
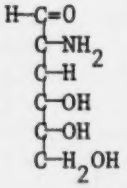
REFRACTORY ORGANICS

Effluents from activated-sludge treatment contain significant amounts of refractory organics, including SON. A clear definition of refractory organics is needed, as is a description of the characteristics which make organic molecules refractory.

Definition

Recalcitrant, refractory, persistent, residual, and non-degradable are terms commonly used to describe organics that are difficult, if not impossible, for microorganisms to metabolize. Hence, such compounds can persist in the environment for relatively long periods of time. Refractory materials may be

TABLE 53. BIOLOGICALLY PRODUCED SON COMPOUNDS

Class	Structure	Percent of Bacterial Cell Weight [77,78,79,80]
Amino Acids	 $R-\overset{NH_2}{\overset{\vert}{C}}-COOH$	40-60
Proteins, Peptides	 $R-\overset{O}{\overset{\Vert}{C}}-\overset{H}{\overset{\vert}{N}}-\underset{\vert}{\overset{\vert}{C}}-R'$	
Enzyme	large proteins	
Amine	$R-NH_2$, $Ar-NH_2$	-
Pyrimidine (heterocyclic N)		-
Purine (heterocyclic N)		-
Nucleoside	Purine or pyrimidine bonded to a 5-carbon sugar	
Nucleotide	Nucleoside bonded to a PO_4^{-3}	-
RNA	Polynucleotide; sugar monomer is ribose	2-40
DNA	Polynucleotide; sugar monomer is deoxyribose	1-5
Hydroxamic Acids (ionophores)	 $R-\overset{O}{\overset{\Vert}{C}}-\overset{O^-}{\overset{\vert}{N}}-R'$	-
Amino Sugars	 $H-C{=}O$ $C-NH_2$ $C-H$ $C-OH$ $C-OH$ $C-H_2OH$	-

where R,R' = H or an aliphatic carbon structure, and
AR = an aromatic structure.

degraded in the environment over periods measured in months, years, or centuries, and by physical or chemical means, as well as biologically. For those concerned with removal of organics from wastewaters, refractory generally refers to materials that are not degraded to a significant extent by well acclimated and operated biological treatment systems. Such materials subsequently degrade slowly, if at all, upon discharge to receiving waters.

Factors Affecting Recalcitrance

Alexander has reviewed and summarized reasons for the failure of microorganisms to degrade organic materials [55,56,57]. These are summarized, with additional support information, in the following.

Inherent Molecular Characteristics--

Certain molecular characteristics make some molecules more resistant to biodegradation than others, but information about specific mechanisms involved is limited. Molecular properties that may affect biodegradability include molecular size, length of chain, stereochemistry, and type, number, and position of substituents in the molecule [45,55,56,57,58,59].

Unsaturated hydrocarbons are degraded more readily than corresponding saturated compounds, most likely due to the more reactive nature of the double bond. In general, branching of aliphatic compounds and substituent chains on aromatics reduces biodegradability, the effect being dependent on the position, extent, and type of branching. The presence of terminal quaternary groups or non-alkyl substituents may markedly affect degradability, especially if degradation proceeds by α- or β-oxidation [55,60]. Enzymes may be unable to contact reactive moieties such as $-NH_2$, $-OH$, $-COOH$, and others to initiate degradation due to unfavorable molecular size, shape, or stereochemistry, thus increasing the recalcitrance of some molecules [45,56,61].

There are data suggesting that substitution of a carbon in a chain with nitrogen, oxygen, or sulfur ($-\overset{|}{\underset{|}{C}}-\overset{|}{N}-\overset{|}{\underset{|}{C}}-$, $-\overset{|}{\underset{|}{C}}-O-\overset{|}{\underset{|}{C}}-$, $-\overset{|}{\underset{|}{C}}-S-\overset{|}{\underset{|}{C}}-$) reduces degradability [56]. Addition of halogens, nitro, or methyl groups imparts resistance to some compounds. On the other hand, addition of $-NH_2$, $-OH$, $-COOH$, and $-CH_3$ to the benzene ring has been shown to render it more degradable [59]. Disubstituted benzene compounds with substituents in the meta position are generally more refractory than ortho- and para-substituted molecules. Also, tri-substituted benzene derivatives degrade at rates slower than those for disubstituted derivatives, which are in turn more refractory than the mono derivatives [45,58,59]. There are notable exceptions to these last two observations.

From the preceding discussion, one could formulate the general conclusion, with exceptions, that molecular recalcitrance increases with increasing molecular complexity, that is, with increasing number and/or types of bonds.

Environmental Factors--

Common environmental factors such as pH and temperature can affect biodegradability if not in ranges optimum for microbial growth [55,56,57]. Lack of an appropriate terminal electron acceptor, or absence of any of a number of essential nutrients such as nitrogen, phosphorus, iron, trace metals, organic growth factors, etc., will render molecules refractory [55]. Toxic materials must be absent [55]. During activated-sludge treatment of domestic wastewaters, these factors are not likely to have a major effect on recalcitrance of effluent organics.

In biological waste-treatment systems, operation must insure that organisms capable of degrading a material of interest are not washed from the system before degradation can be accomplished. Recent studies have indicated that selection of θ_c (solids retention time), which is related to microorganism growth rate, directly affects the concentration of refractory material remaining after activated-sludge treatment [63,64,65,66].

Enzyme-Related Factors--

Since enzymes initiate and catalyze degradation sequences, enzymes capable of attacking the organic molecule and subsequent degradation products must be present, or inducible, and free of inhibition and repression. Enzyme inhibitors may slow the rate of degradation, thus making degradable organics appear relatively refractory. For some organics, no enzyme may exist for initiating the degradation sequence, thereby making the molecule recalcitrant [57]. Certain organics act as catabolic repressors to enzymes required for degradation of other organics [56,67,68,69], and when present, degradation will not take place. Enzymes from several organisms may be required for degradation of a particular organic, and all must be present to insure degradation. If the enzyme system responsible for breakdown is intracellular, the compound must be able to penetrate the cell wall or it will remain undegraded. Apparently, any number of factors can affect enzyme capability and activity, and in this manner contribute to the recalcitrance of organics.

Lack of Sufficient Carbon or Energy for Growth--

The absence of sufficient exogenous carbon for growth and energy may contribute to the recalcitrance of otherwise degradable substances. Addition of a utilizable carbon source to a medium containing a hitherto refractory organic caused degradation of the refractory organic concomitant with utilization of the added carbon source [56,93]. This phenomenon, called co-metabolism, may offer a means of biologically removing otherwise refractory materials.

Low Substrate Concentration--

Degradable materials may not be broken down if they are present in concentrations too low to provide sufficient energy or carbon for cell growth [55,56,57,94]. Thus, if an activated-sludge effluent contained a wide range of degradable substances, but each in concentrations too low to support growth, the substances may be classified as "refractory." Concentration of soluble effluent organics resulted in additional removal [62], which supports the occurrence of the phenomena.

Chemical Reactions that Increase Recalcitrance--

Organics can undergo chemical reactions in the environment which make them less susceptible to degradation. Complexing with other refractory compounds such as lignin, tannins, and polyaromatics reduces degradability [56, 57], as does formation of inorganic complexes with clays and various metals [55,56]. Humic substances, highly refractory organic polymers containing some nitrogen, are formed from simple, degradable precursor molecules excreted by microorganisms [95,96,97]. Condensation reactions of amino acids and simple carbohydrates form refractory nitrogenous polymers (termed melanoidins), including heterocyclic nitrogen compounds, via a mechanism called the Browning reaction [40,41]. Reactions such as these may occur during activated-sludge treatment since a wide variety of inorganic and organic materials are present.

Summary--

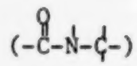
Organic molecules are refractory for a variety of reasons. Environmental factors affecting recalcitrance can be controlled during activated-sludge treatment to minimize adverse effects. θ_c can be manipulated to minimize effluent refractory organics. Factors which cannot be controlled are most likely to be responsible for recalcitrance of effluent organics, including SON. Inherently refractory compounds may be present in the influent or formed chemically or biologically during treatment. Inhibition, repression, and inactivation of enzyme systems increase refractory concentrations. Degradable substances may not be metabolized if sufficient carbon or energy for growth is lacking. If concentrations of degradable materials are too low to support growth, they may not be removed. All or some combination of these uncontrolled factors no doubt contribute to the recalcitrance of effluent organics.

Recalcitrance of SON Compounds

Some organic nitrogen compounds are readily degraded by many microorganisms in order to obtain energy, carbon, and/or nitrogen. Urea is readily degraded by bacteria via the enzyme urease. Free amino acids are metabolized by oxidative, reductive, or hydrolytic deamination reactions. Proteins are broken down to simpler peptides, and finally to amino acids through a series of hydrolytic cleavages catalyzed by several proteinase enzymes. Thus, effluents from activated-sludge treatment contain low concentrations of these materials.

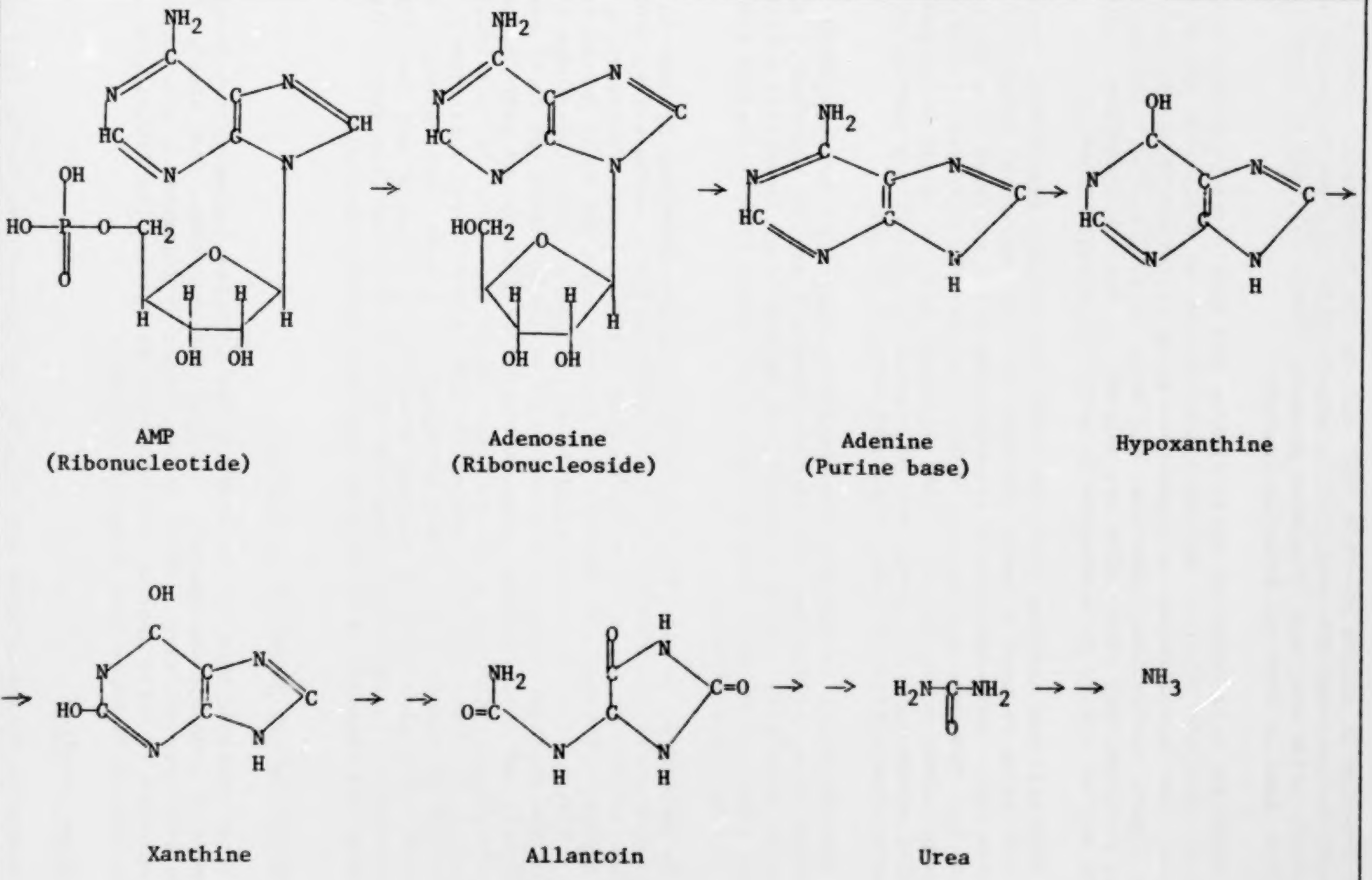
Nucleic acids (DNA and RNA) and their precursors containing nitrogen (nucleotides, nucleosides, purines, and pyrimidines) are degraded at varying rates, to different extents, and accumulate at varying levels in fluids surrounding bacterial cultures and in biological reactors operating under a variety of conditions [61,98,99,100,101,148,149]. Nuclease enzymes aid in the breakdown of DNA and RNA to nucleotides and nucleosides, with DNA the more resistant of the two [61,148]. Nucleotides and nucleosides can be degraded to purines, pyrimidines, and related heterocyclic nitrogen compounds. These compounds may be further metabolized yielding a variety of products (Table 54), such as hypoxanthine, xanthine, uric acid, allantoin, urea, and finally, ammonia [98]. Nucleic acids and their degradation products exhibit varying degrees of biodegradability, making it likely that such materials will be present in activated-sludge effluents.

There is evidence indicating that carbon-nitrogen-carbon bonds $(-\overset{|}{\underset{|}{C}}-\overset{|}{N}-\overset{|}{\underset{|}{C}}\ )$, both in aliphatic and heterocyclic structures, are quite resistant to degradation [56,201]. This is to be distinguished from the peptide bond



$$(-\overset{\overset{\displaystyle O}{\|}}{C}-\overset{|}{N}-\overset{|}{\underset{|}{C}}-)$$

TABLE 54. TYPICAL NUCLEIC ACID DEGRADATION PRODUCTS



AMP (Ribonucleotide) → Adenosine (Ribonucleoside) → Adenine (Purine base) → Hypoxanthine → Xanthine → → Allantoin → → Urea → → NH_3

which contains an oxygen molecule that adds to its reactivity. Carbon-nitrogen-carbon bonds are contained in heterocyclic nitrogen compounds such as nucleic acids and their breakdown products (Table 54), and in condensation products, such as from the Browning reaction.

Bacterial cell walls are quite stable, and must first be solubilized by means of lysozymes prior to enzymatic degradation, at rates that may be quite slow, to less recalcitrant, nitrogen-containing precursor molecules such as amino sugars, nucleotides, proteins, and amino acids [61,206]. The nucleotides, proteins, and amino acids are degraded as described earlier. Amino sugars are not likely to accumulate in activated-sludge effluents [5].

Aliphatic and aromatic nitriles (-C≡N) are readily degradable, the nitrogen first being reduced to amino nitrogen and then removed by hydrolytic cleavage [58]. Mono-substituted nitrosobenzenes are degraded in a similar manner, but rates are considerably slower than for the nitriles or corresponding amino substituents [45,58]. Such compounds are not likely to be found in domestic wastewaters and should not be present in significant quantities in effluents from activated sludge treatment of such waters.

Apparently due to inherent recalcitrant properties, di-substituted nitrosobenzenes, toluidines, and phenylenediamines are not degraded by microorganisms [45,58,59]. These compounds are generally of industrial origin and are not likely to be major constituents in effluents from biological treatment of domestic wastewater.

To summarize, no one class of organic nitrogen compounds present in domestic wastewater, or produced by microorganisms (i.e., amino acids, proteins, purines, nucleic acids, etc.) exhibits what could be termed "outstanding recalcitrance." However, it appears that nucleic acids and their myriad degradation products may comprise a significant portion of activated-sludge effluent SON due to their ubiquitous presence in bacterial cells (up to 45 percent by weight), and their varying rates, extents, and pathways of degradation. Small amounts of amino acids and proteins can be expected. If individual concentrations of these and other degradable SON compounds are sufficiently low, or if there is insufficient carbon or energy for growth, these normally biodegradable molecules could be present in secondary effluents and classified as refractory. In addition, refractory SON may be formed from degradable molecules via chemical or biological reactions during treatment.

SON PRODUCTION AND EXCRETION BY BACTERIA

It is possible that SON is produced and excreted during activated-sludge treatment of domestic wastewaters. Support for this comes from studies conducted with pure cultures. While extrapolation of information obtained with pure cultures to heterogeneous activated-sludge systems has limitations, it can be helpful in explaining observed behavior.

Mechanisms Involved

Excretion of metabolites and cellular contents occurs during cell lysis, or by membrane transport. Membrane transport may take place by one of three mechanisms [150]:

1. Simple diffusion: sometimes termed leakage; requires no energy input; results in equilibration of the compound across the membrane;
2. Facilitated diffusion: accomplished by an interaction with some component of the membrane, such as a permease system; requires no energy input; results in equilibration of the compound across the membrane;
3. Active facilitated diffusion: carrier-mediated process; requires energy input; may occur against or with a concentration gradient.

All three mechanisms may be involved to different degrees depending on factors discussed below.

Factors Affecting SON Production and Excretion

The following list of factors affecting SON excretion is not all-inclusive, and individual factors are not mutually exclusive. The list contains factors thought to be relevant for activated-sludge cultures. Under some conditions, one factor may control excretion, but under most conditions, several factors would no doubt be involved since activated sludge represents a complex, heterogeneous populaton of organisms.

Concentration Gradient--

Organisms are known to excrete organics via diffusion and facilitated diffusion to establish a concentration equilibrium across the cellular membrane [150,151,152]. This type of excretion would be expected when organisms are diluted, the dilution causing a decrease in the concentration of external organics surrounding the cell, thus establishing a concentration gradient. A listing of organic nitrogen compounds excreted in response to a concentration gradient could not be found.

Starvation--

Bacteria excrete organics during starvation, a condition that exists when substrate is essentially gone and cells must obtain energy for maintenance by endogenous respiration or metabolism of intracellular components [99,153,157]. Cell lysis is not considered to be a major factor during starvation, and excretion is via the diffusion transport mechanisms described earlier [109].

The major excretion products observed during starvation are the degradation products of RNA [99,100,109,153,157,177,178]. RNA is an attractive endogenous metabolite since it is present in large quantities in the cell and contains a ribose (sugar) moiety that can be used as an energy source. Cells metabolize the ribose and excrete the associated purine or pyrimidine [99, 100,177,178]. Soluble purines and pyrimidines, and, to a lesser extent, nucleotides and nucleosides, thus accumulate in the liquid surrounding starving cells. Proteins and free amino acids are also excreted but in lower concentration [99,100,112,157]. Cellular DNA is not degraded, and so its degradation products are not observed [99,153].

Presence of an Energy Source--

The presence of an exogenous energy source can stimulate excretion of organics [113,114,115]. Release is energy dependent, and as such, takes place by active facilitated transport. Appearance of the excreted metabolite in the culture may be quite rapid, taking place within a few minutes after substrate addition [113]. Excreted organic nitrogen compounds observed include RNA, nucleotides, proteins, and amino acids [113,115,148].

Substrate-Accelerated Death--

Addition of a carbon and energy source to bacteria starved for carbon and energy accelerates death of the bacteria, a phenomenon called "substrate-accelerated death" [99,116,117,118,189]. Death is defined as the inability to grow in the presence of a utilizable substrate. Cell lysis does not occur. Concentrations of soluble excreted metabolites (RNA degradation products) were reported greater during substrate-accelerated death than during starvation [189]. In another study [116], concentrations were similar but characteristics were different. From reported data, it could not be determined what portion of the excreted metabolites resulted from the increased number of non-viable (dead) cells, and what portion from the remaining viable cells. Regardless, "substrate-accelerated death" results in excretion of organic nitrogen.

Availability of Required Nutrients--

If essential nutrients are absent, or in low concentrations, organics may be excreted [9,118,190,191]. Ionophores, organic nitrogen-containing compounds responsible for chelating various metal ions needed by the cell for transport into the cell, are excreted by bacteria [9,190]. Larger quantities of ionophores are excreted in metal-poor environments, and they are apparently produced and excreted as required by the organism. Other nutrient deficiencies (nitrogen, phosphorus) may result in the production and excretion of organic matter [118,191]. Excretion due to nutrient deficiency is not likely to be a major factor during activated-sludge treatment of domestic wastewaters which are expected to contain sufficient nitrogen, phosphorus, and trace nutrients.

Environmental Stress--

Excretion of organic nitrogen compounds due to environmental stresses such as extreme temperature changes and osmotic shock has been observed [192,193,194,195]. Compounds studied were enzymes and proteins.

Effect of Bacterial Growth Phase and Growth Rate--

Excretion of metabolites to form SON during bacterial growth may result from a combination of mechanisms discussed above, and/or by factors related specifically to growth.

The different major phases of growth are logarithmic, declining, stationary, and death, each defined as follows:

> Logarithmic phase: bacteria growing exponentially; growth rate constant and limited by regeneration time of the organisms and their ability to process substrate;

Declining phase: growth rate decreasing; substrate concentration now limiting growth;

Stationary phase: growth rate essentially zero; starvation conditions prevailing;

Death phase: growth rate negative; during initial stages, cells starving and losing viability, but not lysing; during latter stages, lysis occurs.

More organic nitrogen is produced and excreted during log growth than during stationary growth [32,101,113,148,196,197,198]. The internal mass of DNA, RNA, protein, and free amino acids per cell increases during logarithmic growth. As a result, excretion of these materials and their degradation products by energy-independent diffusion is expected since a higher concentration of the material is present within the cell. In addition, organisms produce and utilize more energy during logarithmic growth [113,114]. This excess energy can be dissipated by increased excretion (energy-dependent, active, facilitated diffusion) or organic nitrogen metabolites no longer needed by the cell [113,115]. Death of cells may occur concurrently with growth, but during log growth, production and excretion of metabolites as a result of growth far exceeds the quantity of material released by cell lysis [101,113,148].

During the stationary phase, starvation conditions prevail, and excretion occurs as described previously (Starvation Factor). As starvation continues, organisms begin to die, that is, lose viability. Organics released by nonviable cells, including nucleotides and their degradation products, can be used as carbon and energy sources by viable cells [199]. Cell lysis occurs in the latter stages of the death phase, releasing organic nitrogen compounds such as nucleic acids, nucleotides, nucleosides, and proteins [32,148,149,196,197]. Soluble material released during cell lysis is fairly degradable and does not accumulate in large quantities in the medium [101,203,204].

Growth rate is related to phase of growth as described above, and therefore, may similarly affect the excretion of organic nitrogen metabolites. Substrate concentration determines growth rate. The relationship, developed by Monod and modified by others, is [200,205]:

$$\mu = \frac{Y\ k\ S}{K_s + S} - b \qquad (6)$$

where μ = net growth rate (time^{-1}),

Y = growth yield coefficient (mass/mass),

k = maximum rate of substrate utilization per unit weight of microorganisms (time^{-1}),

S = substrate concentration surrounding the organisms,

K_s = half-velocity constant (mass/volume), equal to the substrate concentration where substrate utilization is 1/2k, and

b = microorganism decay rate (time^{-1}).

Examination of Eq. 6 shows that at high growth rates, the relative effect of organism decay is small, and a higher proportion of the cells will be growing logarithmically. In light of previous discussion, it might be postulated that increasing growth rate may result in higher concentrations of organic nitrogen metabolites being excreted. This needs to be evaluated.

Summary

SON is produced by bacteria; the major classes produced are nucleic acids, proteins, and their respective degradation products. Factors affecting production and excretion include: (1) concentration gradients, (2) starvation, (3) presence of an energy source, (4) substrate-accelerated death, (5) availability of nutrients, (6) environmental stress, and (7) phase and rate of growth. None of these individual factors is likely to be the dominant factor controlling SON production by activated sludge; all may be involved.

SUMMARY AND BASIS FOR EXPERIMENTAL APPROACH

The concentration of SON in effluent from activated-sludge treatment of municipal wastewaters varies from about 0.8 to 2 mg/l (Section 6). The purpose of this study was to determine the characteristics of this material and to evaluate the relative distribution of effluent SON between that formed by the biological treatment process itself and that originally present in the influent stream. This section has discussed factors affecting SON production and consumption, generally by pure cultures of bacteria. Whether these factors are important in mixed activated-sludge cultures, and if so, what contribution each factor makes to the total effluent SON from secondary effluent are unknown. This experimental study was designed so that the factors which may contribute to effluent SON and which were thought to be of significance in normal activated-sludge operation could be evaluated.

Factors affecting SON consumption (removal) that can be controlled during activated-sludge treatment are environmental factors; the important ones were thought to be detention time and organism concentration. Batch studies with domestic wastewater as the substrate, varying aeration time and activated-sludge MLSS concentration, were used to evaluate these factors and to provide estimates for SON_e (Eq. 2).

The factors which appear of importance in effluent SON production (SON_p, SON_g, SON_d) are: (1) concentration gradients, (2) starvation, (3) presence of an energy source, (4) substrate-accelerated death, (5) nutrient availability, and (6) phase and rate of growth. Factor 1 was evaluated in batch studies to measure the SON initially released upon dilution of concentrated activated sludge (termed Initial Release Studies) as a function of MLSS concentration, culture characteristics, temperature, and substrate. The effect of starvation was evaluated with batch studies, termed Organism Decay Studies, in which activated sludge was diluted with tap water, and aeration time and

MLSS concentration were varied. Factors 3 to 6 are related to the presence of a utilizable substrate and were evaluated with batch systems fed various synthetic wastes (Synthetic Feed Studies). Aeration time, SCOD, MLSS, NH_3-N, and substrate type were the variables evaluated. To obtain additional data on the effect of phase and rate of growth, mixed cultures were maintained by semi-continuous feeding to simulate activated-sludge operation, and were fed a synthetic organic waste which contained little SON so that SON production could be better evaluated.

LABORATORY ACTIVATED-SLUDGE CULTURES

Apparatus

Semi-continuous feed, laboratory activated-sludge (AS) cultures were grown in two 9-liter pyrex glass bottles as pictured in Figure 8, and were operated in a walk-in constant-temperature incubator maintained at 20 ± 1°C. The magnetic mixers used increased culture temperature to about 21°C. Pressure control valves permitted close control over the flow rates of carbon dioxide and air.

Attached microbial growth was kept at a minimum by vigorously shaking the bottles by hand daily, and scraping excess growth off container walls with a brush monthly. Porous diffusers were cleaned frequently with chromic acid cleaning solution.

Feed System

The two activated-sludge cultures were grown with the synthetic substrate or waste listed in Table 55. A glucose-acetate mixture was selected to provide a relatively simple carbon source containing no SON, which would permit development of a somewhat heterogeneous microbial population. Stock feed solutions of glucose, acetate, NH_4Cl, K_2HPO_4, and Fe-Co were made separately in deionized water and stored at 4°C. AS Culture 1 was started on October 15, 1975 and AS Culture 2 on March 24, 1976.

Once the systems reached steady-state operation, COD/N ratios in the feed ranged from 24 to 30 and N/P was kept constant at 5. The quantity of nitrogen added was varied in an attempt to keep effluent inorganic nitrogen relatively low to minimize the chances for nitrification, since an interference with SON analysis was observed when NO_3^--N exceeded 10 mg/l (Appendix B). Addition of Fe and Co was commenced 133 days after the start of AS Culture 1 in an effort to control sludge bulking, and were included in the feed solution of AS Culture 2 at all times. The pH was controlled in the 7.0-7.5 range by mixing pure CO_2 with the air flowing into the cultures (about 1-2% CO_2).

AS cultures were started by adding 30 ml of one-day-old settled sewage from the Palo Alto, California, Regional Water Quality Control Plant to 6 liters of feed (Table 55). After initial start-up, the feeding procedure included wasting one of the six liters of mixed liquor (to make $\theta_c = 6$ days), followed by one hour of settling, wasting 3 liters of supernatant liquid

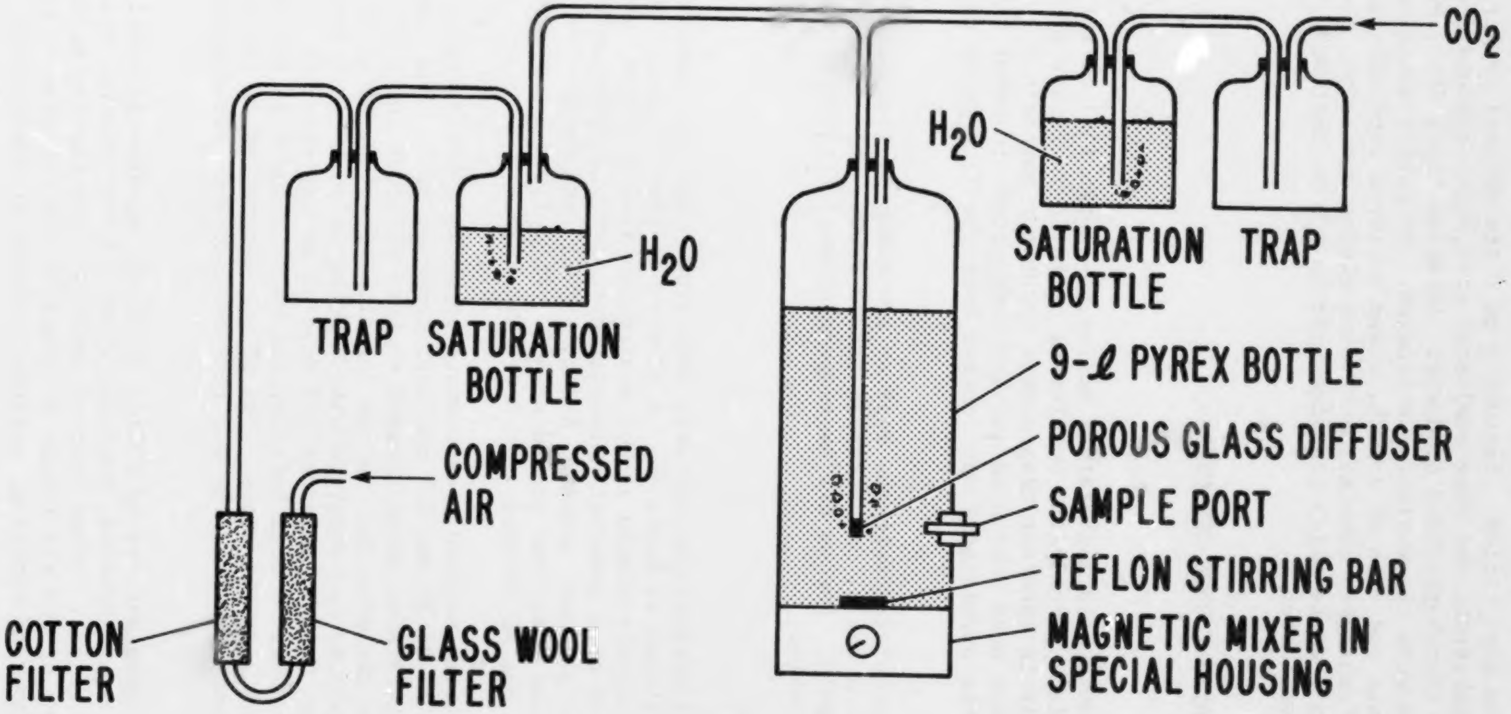

CO₂
H₂O
SATURATION BOTTLE
TRAP
TRAP
SATURATION BOTTLE
H₂O
9-ℓ PYREX BOTTLE
POROUS GLASS DIFFUSER
SAMPLE PORT
TEFLON STIRRING BAR
MAGNETIC MIXER IN SPECIAL HOUSING
COMPRESSED AIR
COTTON FILTER
GLASS WOOL FILTER

Figure 8. Laboratory activated-sludge system.

TABLE 55. COMPOSITION OF SYNTHETIC WASTE

Compound	Concentration
Glucose	0.75 g/l as COD
Na Acetate	0.75 g/l as COD
NH_4Cl	50-62 mg/l as N
K_2HPO_4	10-12 mg/l as P
$FeCl_3 \cdot 6H_2O$	0.15 mg/l as Fe
$CoCl_2 \cdot 6H_2O$	0.15 mg/l as Co
Dilution water	Stanford tap water

(culture effluent) and addition of 4 liters of feed solution. The mixture was aerated for 23 hours and the feeding procedure repeated. This semi-continuous feeding simulates a plug flow activated-sludge operation.

Effluent or mixed liquor were periodically monitored for pH, mixed liquor volatile and suspended solids concentration (MLSS and MLVSS), SCOD, SON, NH_3-N, NO_2^--N, and NO_3^--N. Samples for SCOD and SON analyses were first filtered through a glass fiber filter (Reeve Angel 934 AH), and then through a 0.45μ cartridge filter (Pall DFA 3001 AXA). SON analysis was by the Kjeldahl method [3]. Microscopic observations of the culture were made approximately once a month and dissolved oxygen was checked infrequently. SON of the feed solution and tap water were determined from time to time.

BATCH EXPERIMENTS

This section describes common apparatus and experimental procedures used for all batch studies. Details on individual experiments are presented later.

Apparatus

Most batch studies were conducted in 1-liter or 4-liter aspirator bottles. One study was conducted using a 9-liter purex bottle identical to that depicted in Figure 8. All glassware was acid-washed prior to use.

The batch systems were essentially identical to the activated-sludge system. Liquid temperatures were maintained at 21 ± 1°C during all studies and pH control was accomplished using an air-CO_2 mixture. Liquid volumes used were 750-900 ml in the 1-liter aspirator bottles, 1.0-3.5 liters in the 4-liter aspirator bottles, and 5.5-7.0 liters in the 9-liter bottles.

Procedures

Mixed cultures for the batch experiments were activated sludge taken either from the Palo Alto complete-mix activated-sludge treatment plant, or from one of the laboratory activated-sludge systems. The cultures were settled for concentration and the supernatant liquors decanted or siphoned for subsequent analyses. The cultures were then added to the various temperature-equilibrated and, in some cases, oxygen-saturated substrates to be tested.

Samples were withdrawn at time 0 (within 30 seconds after culture addition) and at specified times for SON, SCOD, MLSS, MLVSS, and in some cases NH_3-N, NO_2^--N, and NO_3^--N analyses. Temperature, pH, and dissolved oxygen were also monitored.

Separation of the soluble fraction was accomplished by one of the following methods:

1. Sedimentation for 5-10 minutes followed by filtering with glass fiber filter (Reeve Angel 934 AH) and final filtration with a 0.45μ Pall DFA AXA cartridge filter, or

2. Centrifugation of 35-ml samples in a bucket-type centrifuge (International Chemical Centrifuge, International Equipment Co., Boston, Mass.) for 5-10 minutes followed by filtration with 0.45μ Millipore filters in syringe cartridges (SX00 02500 Swinnex, Millipore Corp.).

All filters were washed with deionized water prior to use. Table 56 lists the filtration method and SON analytical technique used for the various batch studies.

SON removal studies were conducted using Palo Alto mixed liquor, unfiltered and filtered (Method 1) primary effluent, and in one case, a synthetic waste containing glucose.

SON production was evaluated using laboratory AS cultures and various concentrations and combinations of synthetic waste in tap water. $NaHCO_3$ was added for pH control and feed solutions were saturated with oxygen using the air-CO_2 mixture prior to addition of cultures. Initial release and organism decay studies made use both of Palo Alto and laboratory AS cultures diluted in tap water.

To investigate the effect of extended periods of aeration on release of SON and SCOD during organism decay, two small aerobic digesters were operated using waste laboratory activated sludge from Culture 1. Aeration conditions were identical to those for activated-sludge operation. One digester was maintained at a θ_c of 20 days by daily semi-continuous feeding. An infinite θ_c system was developed by saving waste AS culture sludge on 4 consecutive days and aerating with no daily wasting or additional feeding.

TABLE 56. Filtraton and SON Analysis Used for Batch Studies

Batch Study No.	Bottle Type	Filtration Method	SON Analysis
1	9-liter pyrex	2	Technicon
2-4, 6-7	1-liter aspirator	2	Technicon
all others	4-liter aspirator	1	Kjeldahl

SON REMOVAL

Objectives

The objectives of this phase were to determine the effect of aeration time, sludge washing, initial MLSS concentration, and substrate type on the SON remaining (SON_e) after activated-sludge treatment of a primary treated, municipal wastewater. This would provide insights into the changes occurring in SON during treatment. Batch studies were used exclusively for evaluating the different variables.

Procedures

Statistical procedures required in this study are outlined in Appendix A. Batch studies were conducted using concentrated mixed liquor from the Palo Alto regional treatment plant for seed, and filtered and unfiltered primary effluent and in one case, glucose, as substrate. Concentrated mixed liquor diluted with Stanford tap water generally served as a control. Table 57 summarizes the substrates, filtration techniques, and SON techniques used. pH was maintained between 7.0 and 7.6, and excess air was added so that dissolved oxygen would not be limiting.

The initial concentrations of SON and SCOD in batch experiments were calculated as follows:

$$SON_{oc} = \frac{SON_f(V_f) + SON_{ml}(V_{ml})}{V_f + V_{ml}} \qquad (7)$$

TABLE 57. SUMMARY OF SUBSTRATES, FILTRATION, AND SON ANALYSIS USED DURING REMOVAL STUDIES

Batch Study Number	Substrates	Filtration* Technique	SON Analysis
1	Unfiltered Primary, Tap Water	Centrifugation-Syringe Millipore	Technicon
2	Unfiltered Primary	Centrifugation-Syringe Millipore	Technicon
3	Filtered Primary, Tap Water	Centrifugation-Syringe Millipore	Technicon
4	Filtered Primary, Glucose-Nutrient-Tap, Tap Water	Centrifugation-Syringe Millipore	Technicon

*As described in Chapter 4, using 0.45μ Millipore filters.

where SON_{oc} = calculated initial SON in mg/l,

SON_f = SON of feed solution in mg/l (primary effluent or tap),

SON_{ml} = SON of mixed liquor in mg/l,

V_f = volume of feed solution added in liters, and

V_{ml} = volume of mixed liquor added in liters.

$SCOD_{oc}$ was calculated in a similar manner.

Effect of Aeration Time

Batch Studies 1 and 2 were conducted to evaluate the effect of aeration time on SON and SCOD removal. Results are presented in Figure 9, 10, and 11. In Batch Study 2 the effect of sludge washing on SON and SCOD removal was investigated. For one of the batch units, 6 liters of Palo Alto mixed liquor was washed four times with tap water. This included settling, decanting, and addition of tap water to give a 6-liter volume after each wash.

In all cases, SON decreased in concentration with time until some minimum was reached, after which the concentration increased. At the point of minimum concentration, SON removal was 70 percent in Batch Study 1, and 61 and 65 percent for the unwashed and washed sludges of Batch Study 2, respectively. After the minimum, the increase in SON was dramatic during Batch Study 1 and after one day of aeration reached a concentration greater than originally present in the influent (Figure 9), and more than 4 times the minimum concentration. The increase was not as great during Batch Study 2 (see Figure 11). No explanation for this difference in behavior can be given, and the results of Batch Study 1 could not be duplicated.

SCOD removal paralleled SON removal in both studies. When SCOD reached a minimum value, removals were 75, 64, and 67 percent, respectively, for Batch Study 1 and the unwashed and washed sludges of Batch Study 2. After a minimum SCOD was reached there was a gradual increase in SCOD as aeration time increased, an observation noted by others [62,212,213,214,215,216]. The magnitude of this increase (140 percent) was small compared to the 400-percent increase in SON during Batch Study 1. In Batch Study 2, the increases were around 140 percent for both SON and SCOD. This disparity in behavior during Batch Studies 1 and 2 cannot be explained, but shows that SON and SCOD may not behave similarly.

SON and SCOD were released during organism decay (see control systems, Figures 9 and 10). However, in Batch Study 1 the increases from minimum SON and SCOD values to measured 48-hour values (3.28 and 7.2 mg/l, respectively) cannot be entirely attributed to decay of the initial population since SON and SCOD released from tap water controls were only 1.35 and 4.2 mg/l, respectively. The initial presence of a utilizable carbon source resulted in increased release of SON.

Examination of data presented in Figures 9 and 10 leads to questions

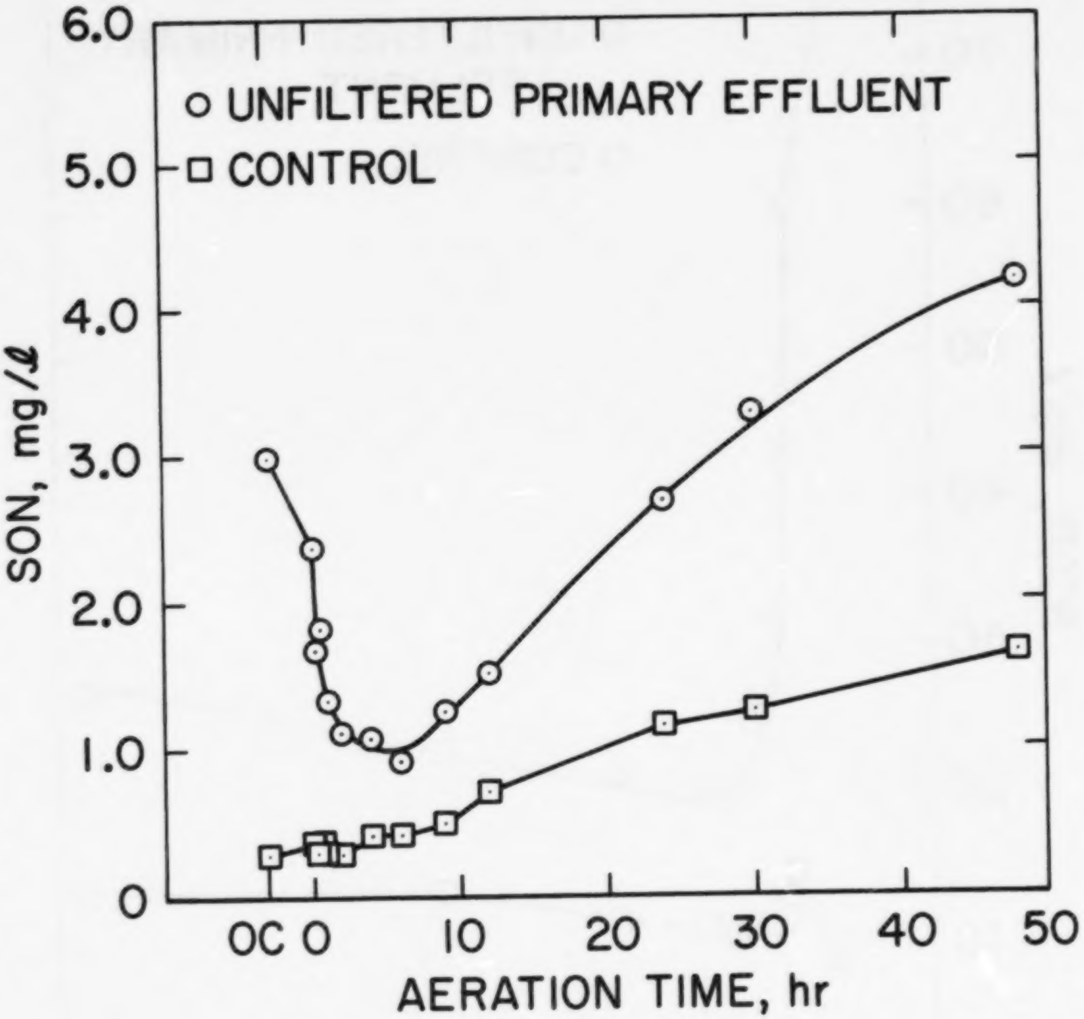


Figure 9. Batch Study 1: Effect of aeration time on SON remaining, $MLSS_{om} \approx 1300$ mg/l.

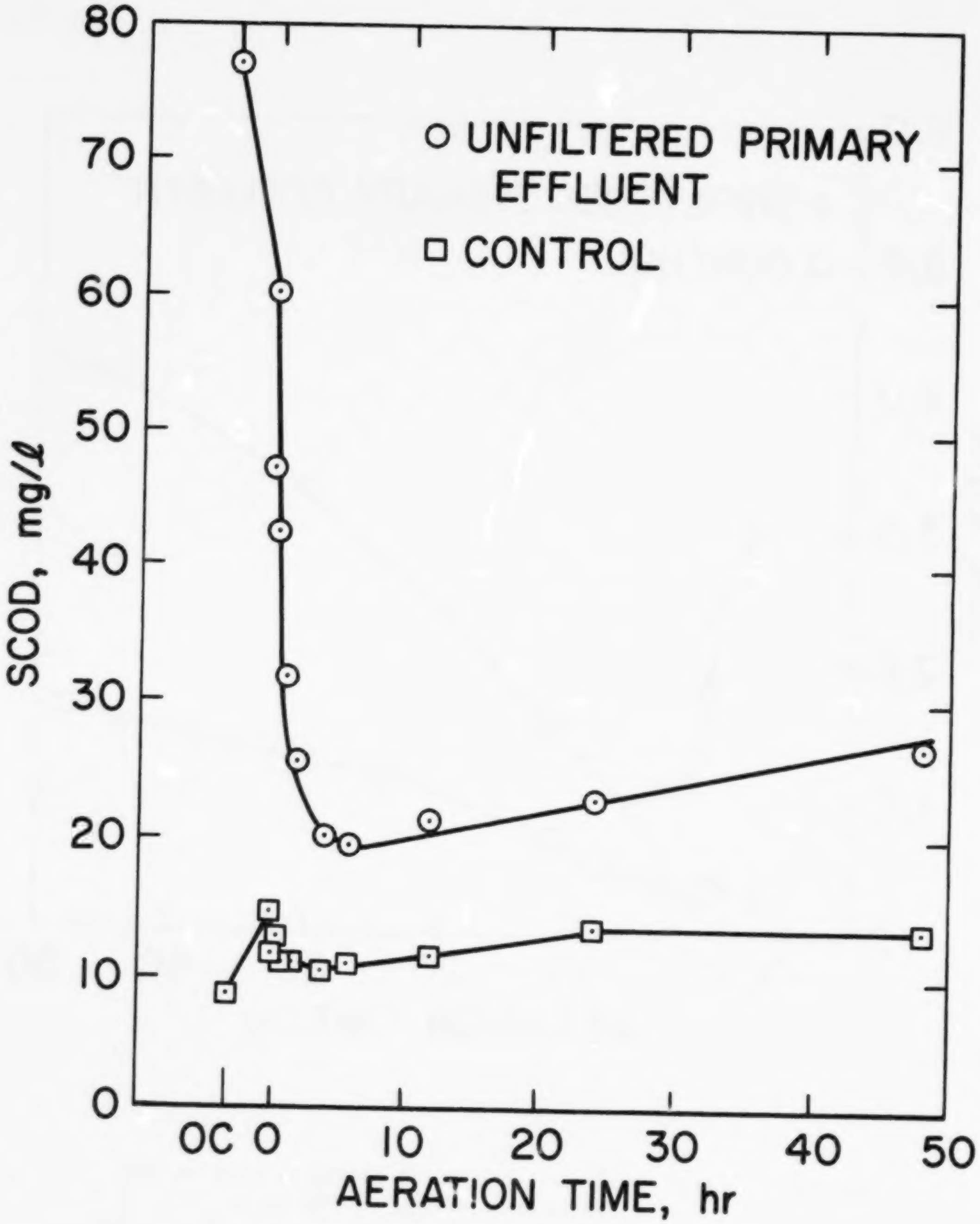


Figure 10. Batch Study 1: Effect of aeration time on SCOD remaining, $MLSS_{om}$ ≈ 1300 mg/l.

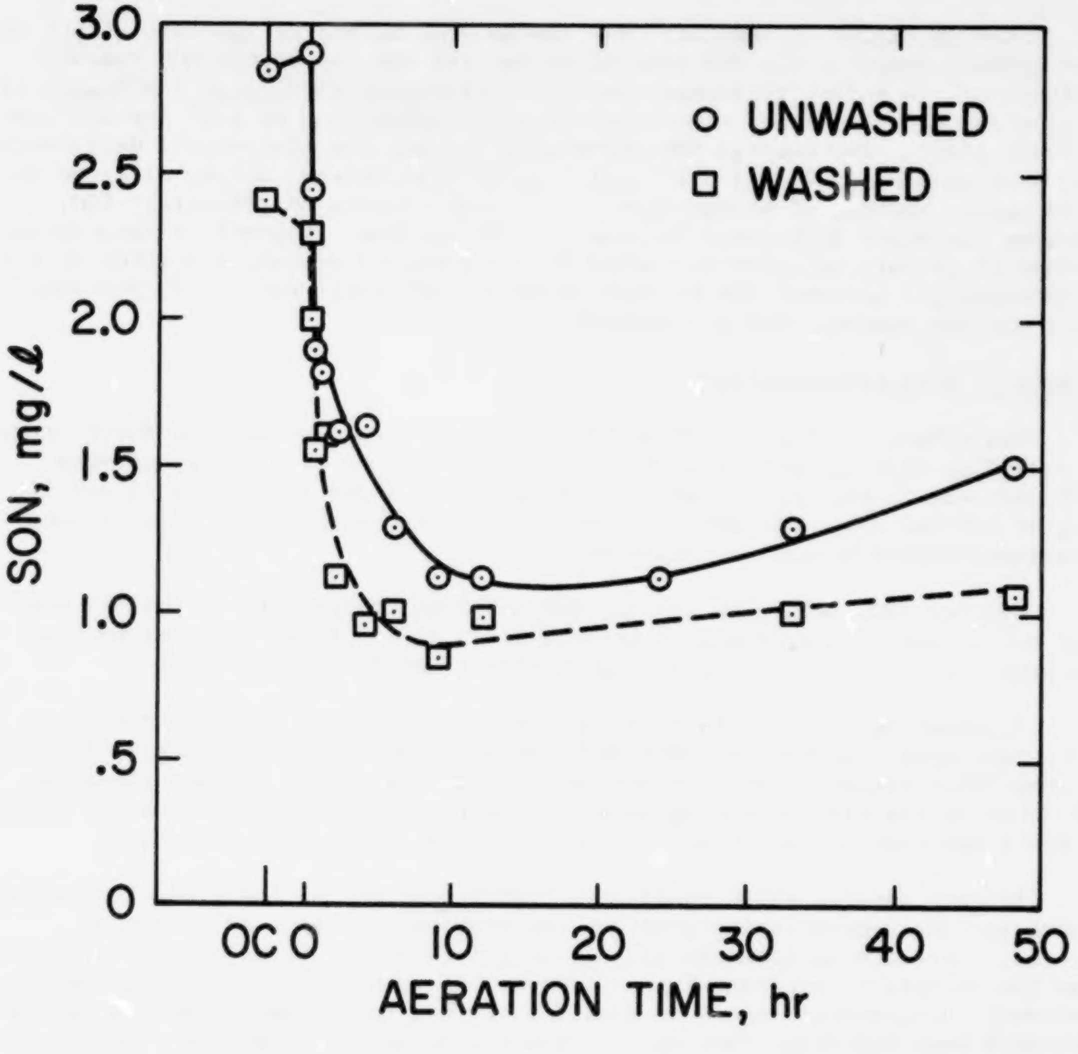


Figure 11. Batch Study 2: Effect of sludge washing and aeration time on SON removal from unfiltered primary effluent, $MLSS_{om}$ = 1200 mg/l.

about differences in characteristics of the SON present after certain periods of aeration. Samples were taken from the control and primary-fed systems at times 0, 6, and 24 hours and subjected to low-seed biodegradation (identified as Biodegradation Study 3) to investigate these differences. Results and their significance are discussed later.

Data in Figure 11 indicate that sludge washing had no apparent effect on the general shape of the SON removal curve, and did not affect SON removal efficiency. A t-test indicated that the differences ($SON_{unwash} - SON_{wash}$) of equivalent aeration times were statistically significant at a 99 percent confidence limit. The average SON difference between the 11 measured data points for each sample was 0.39 ± 0.17 mg/l. Calculated initial values differed by 0.45 mg/l. Washing of sludge resulted in some removal of refractory SON, causing the major difference between the two systems. However, since a larger volume of primary effluent was added to the unwashed system, a portion of the difference (37 percent) can be attributed to additional refractory SON added that was not removed during treatment.

Effect of MLSS Concentration

The effect of MLSS on SON and SCOD removals was evaluated in Batch Study 3 by adding varying amounts of Palo Alto mixed liquor to filtered primary effluent and to tap water controls. Results are shown in Figures 12 and 13. Higher removal rates for SON and SCOD are obtained with higher mixed liquor suspended solids levels, as expected.

For the 1390 mg/l MLSS system, SON and SCOD concentrations reached minimum values and then gradually increased to about 1.5 times the minimum concentrations. These observations agree with those from Batch Study 2.

Examination of Figures 12 and 13, and comparison of SON/SCOD ratios as aeration times increase indicate that SON and SCOD behave differently for the 3 lower MLSS systems; SCOD was removed at a faster rate. SON was produced, as noted by its significant increase (95 percent level of significance) after 6 hours aeration in the 14 and 1.4 mg/l systems, while SCOD decreased.

Primary factors which could have contributed to the SON production noted above include concentration gradient, starvation, presence of an energy source, substrate-accelerated death, and growth rate. Since primary effluent was the substrate, and experimental conditions were controlled to maintain a favorable environment, nutrient availability and environmental stress should not have been important factors. Starvation (organism decay) of a portion of the population may have contributed to SON production because significant quantities of SON were released from control systems. The presence of an energy source can stimulate production by energy-dependent facilitated diffusion, or by substrate-accelerated death. Growth rate remained high during the first 6 hours of aeration since substrate concentration (SCOD) decreased slowly due to low initial MLSS values. These sustained high growth-rate conditions may have stimulated SON excretion. Similarly, SON production was not observed for the two higher MLSS systems because organism concentrations were higher, and SON was removed faster than it was produced and excreted.

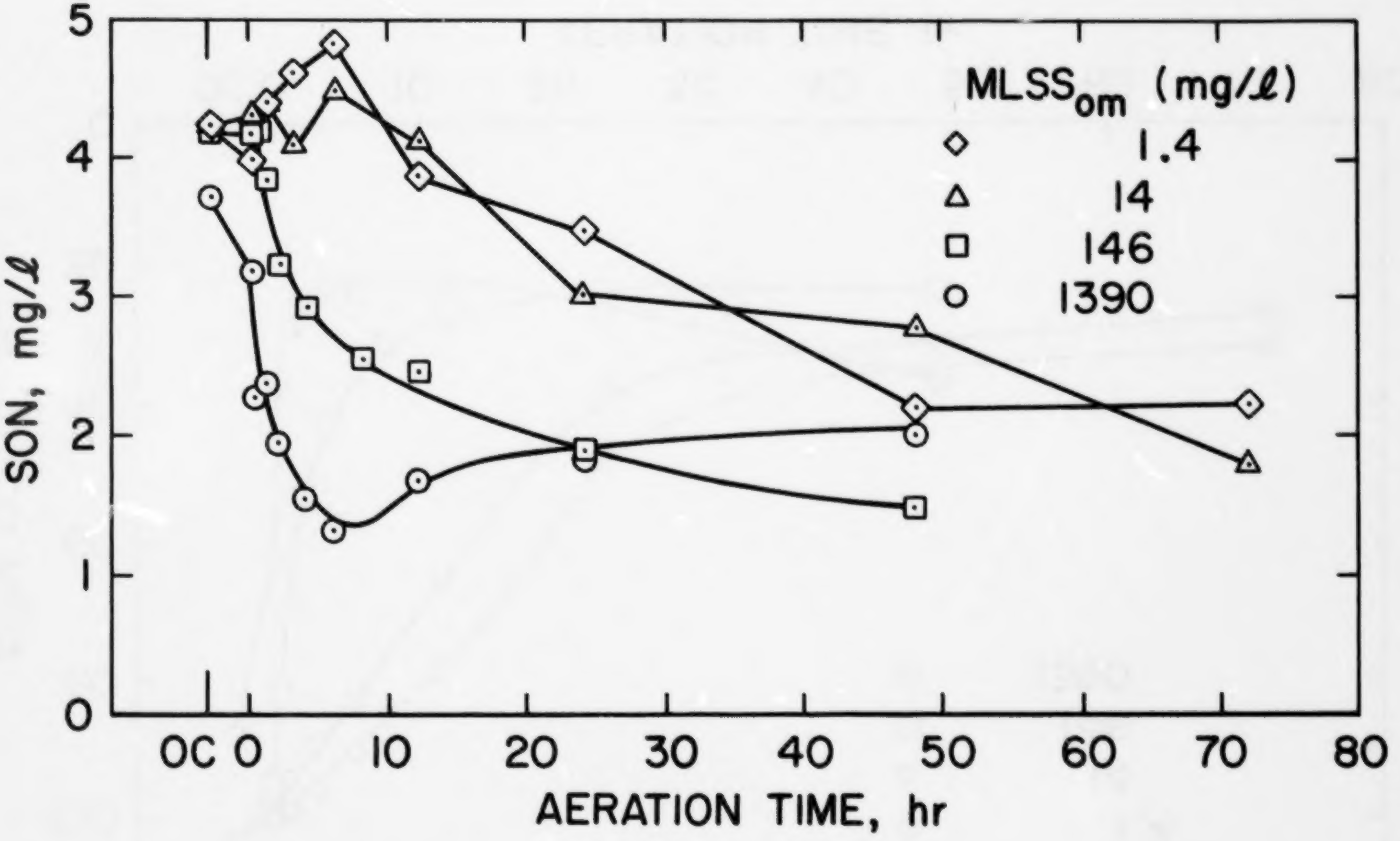


Figure 12. Batch Study 3: Effect of MLSS$_{om}$ on SON removal from filtered primary effluent.

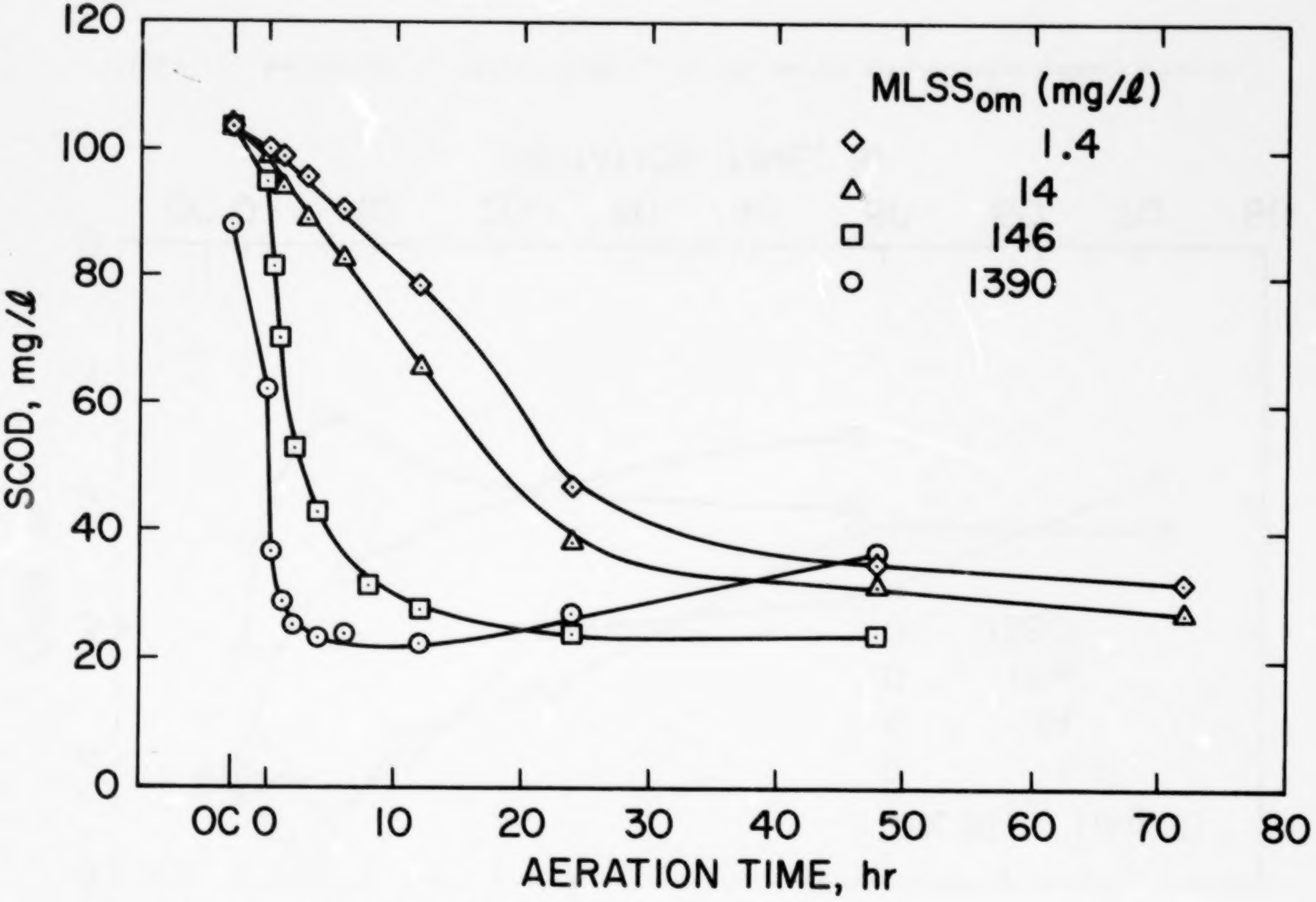


Figure 13. Batch Study 3: Effect of MLSS$_{om}$ on SCOD removal from filtered primary effluent.

Effect on Substrate Type

The previous batch studies used a feed which contained SON and thus made it difficult to ascertain what portion of the SON present after extended aeration periods was attributable to the SON initially added, and what portion was produced biologically during substrate removal. In order to gain more understanding of this aspect, Batch Study 4 was conducted to compare SON concentrations from batch systems fed a non-SON-containing feed and primary effluent.

For the non-SON feed, a glucose-nutrient-tap water solution was used (SCOD = 189 mg/l). Inorganic nitrogen was added in the form of NH_4Cl (SCOD/N = 20) and phosphorus was added as K_2HPO_4 (N/P = 5). Filtered primary effluent and tap water (control) were used to compare SON behavior. Results are shown in Figures 14 and 15.

Removal of primary effluent SON and SCOD was similar to results obtained in Batch Study 3 at approximately equivalent MLSS concentrations. Comparison of the SON concentrations measured after 48 hours aeration with glucose (0.8 mg/l) and with filtered primary (1.62 mg/l) suggests that a significant portion of effluent SON may result from production during substrate oxidation. SON released by organism decay (control system) was 0.73 mg/l after 48 hours, a level not significantly different than observed for the glucose system. These data suggest that the major fraction of the produced SON may result from organism decay. Additional data on this aspect is presented later.

A statistically significant SON peak was observed after 4 hours aeration for both the glucose and control systems. Differences between values measured after 4 hours and the calculated initial value were greater than the 0.28 mg/l required for a 95 percent level of significance (Appendix A). Substrate related factors, such as presence of a utilizable energy source, substrate-accelerated death, and growth rate could explain the SON production by the glucose system. However, these are not the only factors involved since the control system (starvation conditions) exhibited similar peak behavior. Peak SON concentration (0.75 mg/l) was significantly less than for the glucose system (1.12 mg/l), but the shape of the SON curves were similar. Additional information on these phenomena is given later.

Summary

Batch removal studies with primary effluent gave SON and SCOD removals of 60-70 percent, results consistent with those reported in full-scale activated-sludge plants (Section 6). The data provide an estimate that effluent SON (SON_e) equals 38 ± 6 percent of the influent SON (SON_i), or 1.40 ± 0.46 mg/l for the 8 samples tested. There is an aeration time at which SON concentration reaches a minimum and beyond which it increases, primarily due to organism decay. Washing activated sludge removes some refractory SON and thus results in generally lower SON values. Higher MLSS concentrations result in higher SON removal rates.

SON is produced during activated-sludge treatment, and a major portion of that produced may be due to organism decay. SON production during organic oxidation could account for up to 50 percent of secondary-effluent SON (SON_e).

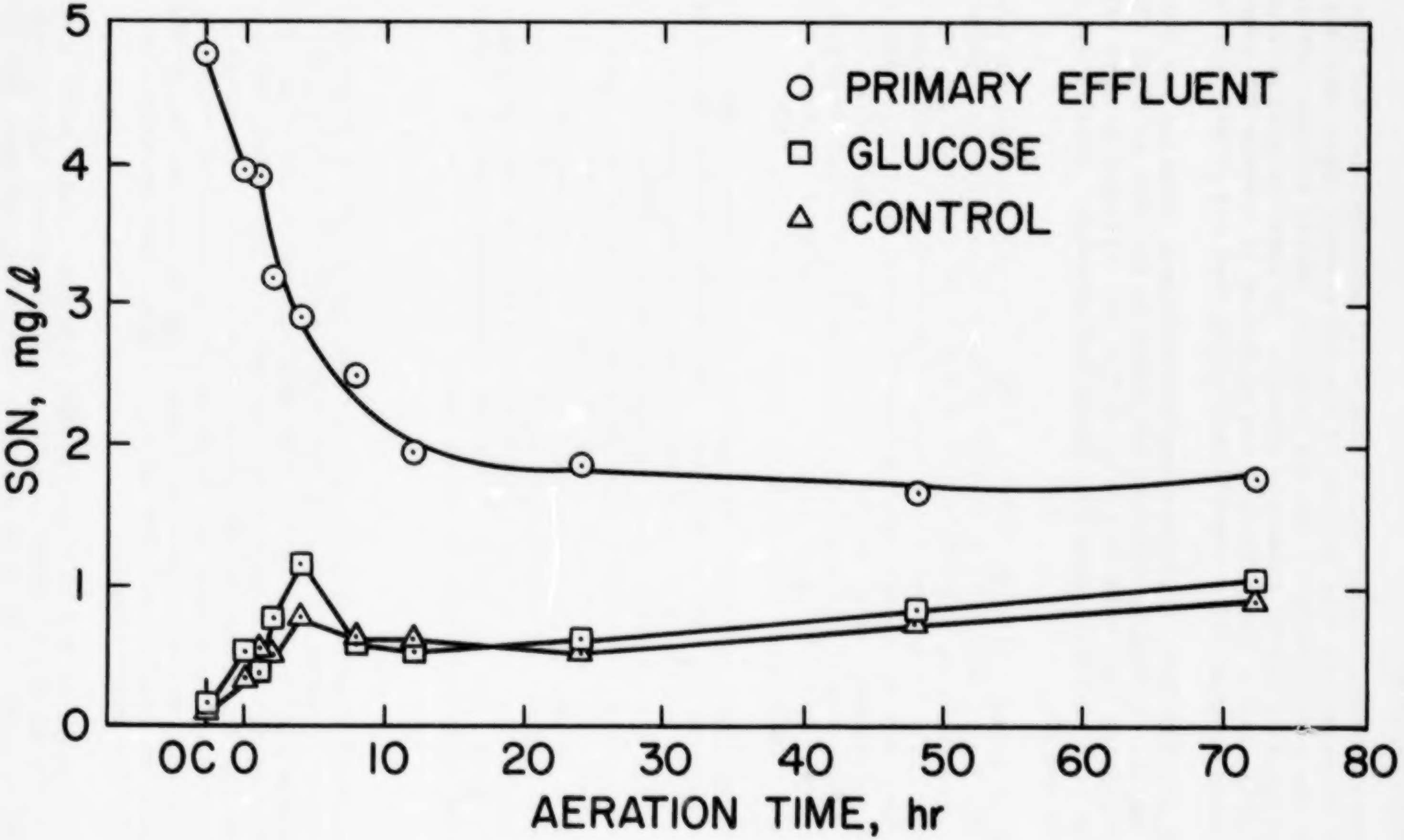


Figure 14. Batch Study 4: Effect of substrate on SON remaining, $MLSS_{om} \approx 180$ mg/l.

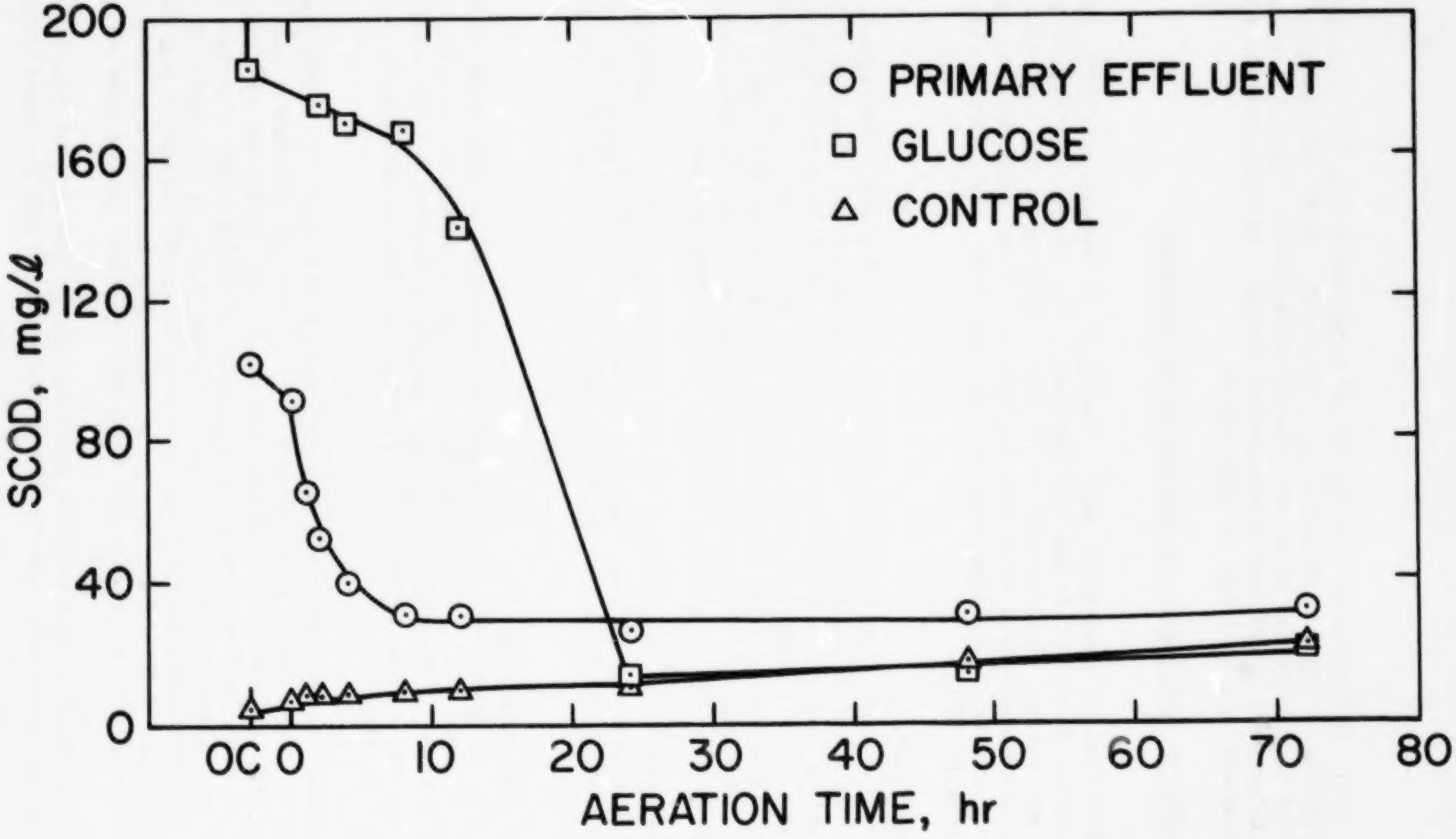


Figure 15. Batch Study 4: Effect of substrate on SCOD remaining $\text{MLSS}_{\text{om}} \simeq 180$ mg/1.

SON PRODUCTION

Objective

The overall objective of the following experiments was to evaluate SON_p, the SON produced during activated-sludge treatment. Both semi-continuous and batch systems were used and were fed low-SON substrates. Specific objectives are given under each individual experimental phase. Statistical procedures used in this study are outlined in Appendix A.

SON Production by Laboratory Activated-Sludge Cultures

Objective--

The objective of this study was to monitor the behavior of SON_p during activated sludge start-up, while the culture passes through different phases of growth, and during steady-state operation. SCOD and other constituents, and culture characteristics were also monitored. These data provide general information about daily fluctuations in SON_p and SCOD, and provide clues about factors affecting SON_p.

Feed Characteristics--

The low-SON feed (glucose plus acetate) described under Experimental Procedures was used as the substrate. Stanford tap water was used for dilution and was found to contain SON with a concentration of 0.05 ± 0.03 mg/l (range: 0.01-0.11 mg/l) for 42 samples analyzed between August, 1975 and September, 1976. During the period from April through the middle of May, the tap water temporarily came from a different source and SON was a bit higher, ranging from 0.11-0.23 mg/l for 5 samples analyzed during this period.

Feed solution SON, containing all the nutrients listed in Table 55, averaged 0.10 ± 0.04 for 21 samples analyzed at various times during the research. Measured feed-solution SCOD averaged 1455 mg/l, 97 percent of the theoretical value calculated.

Activated-Sludge Development--

Laboratory activated-sludge cultures were started as described previously. Behavior of effluent SON and MLSS during start-up is shown in Figures 16 and 17. During start-up, the culture was not purposely wasted. After start-up, a given percentage of the culture was removed each day to maintain a defined solids retention time (θ_c).

SON reached its maximum concentration during the start-up. While still in the start-up phase (Days 13 to 32 and 7 to 23 for AS Cultures 1 and 2, respectively), SON concentration sharply decreased, and continued to decrease as the rate of MLSS increase began to decrease. SON decreased further when culture was wasted for control of θ_c until steady-state values were reached. Values for effluent SCOD ranged from 36-145 mg/l for AS Culture 1 and 26-60 mg/l for AS Culture 2 during the period of sharp SON increase and decrease, and from 10-30 mg/l for both cultures when SON and MLSS values leveled off.

The SON released during culture start-up was primarily the result of excretion of metabolites since the feed contained little SON by comparison.

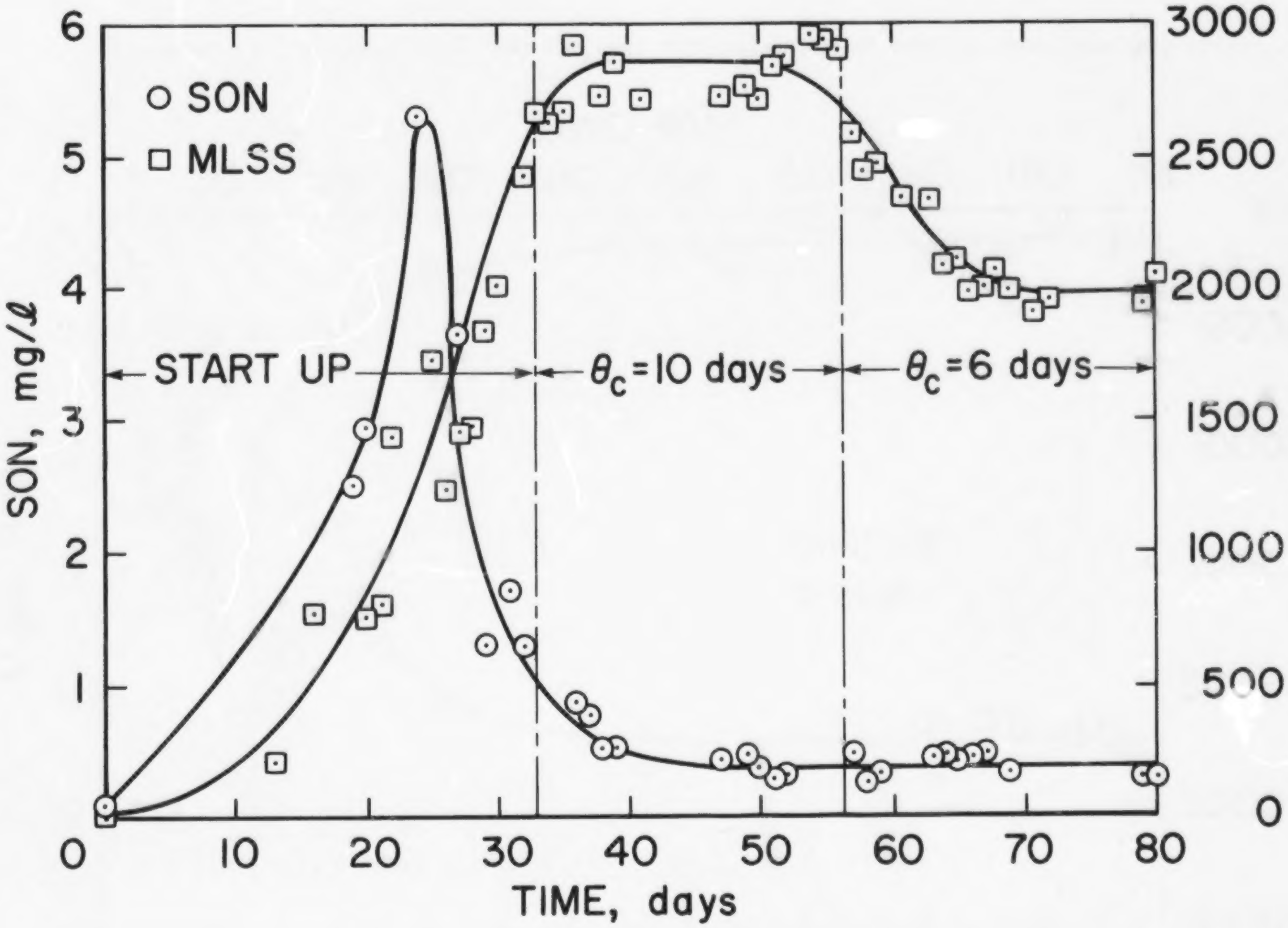


Figure 16. AS Culture 1 start-up: SON and MLSS concentrations vs time of operation.

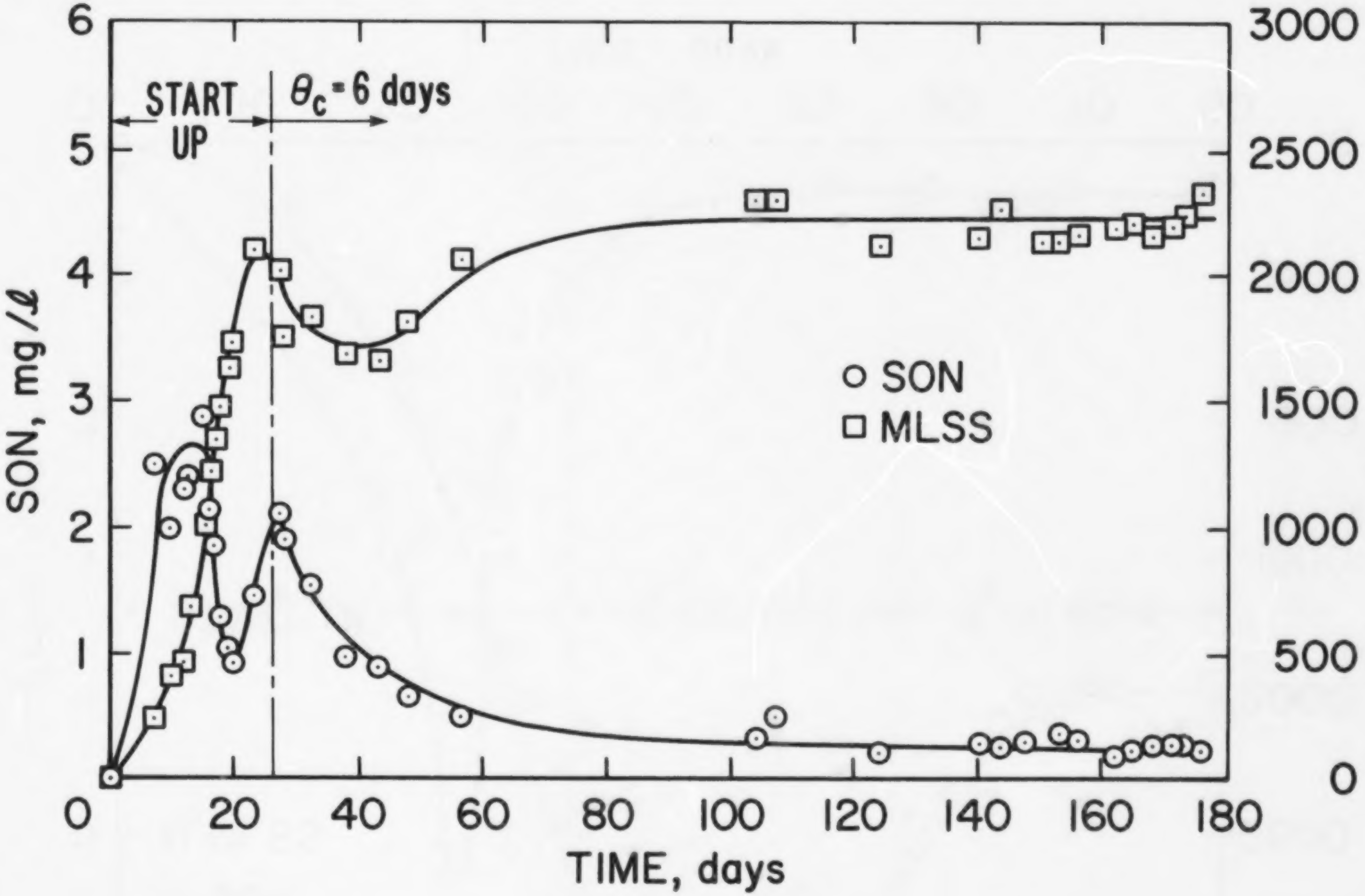


Figure 17. AS Culture 2: SON and MLSS concentrations vs time of operation (all data).

Data in Table 58 indicate that phase and rate of growth did not control the production of SON. SON decreased significantly while still in the logarithmic start-up phase. Comparison of steady-state SON concentration ($\mu = 0.17\ day^{-1}$) with SON concentrations measured during culture start-up ($\mu = 0.15\ day^{-1}$) suggests that growth rate does not control SON production.

Apparently, factors other than rate and phase of growth have a significant influence on SON production. Concentration gradients are not likely to be a factor since produced SON levels were so high. Nutrient availability and environmental stress should not affect production since feed solutions contained most of the required nutrients, and culture environment (temperature, pH, etc.) was controlled. The effect of starvation and substrate accelerated death are not known because it was impossible to determine if starvation conditions existed during the logarithmic start-up phase. The presence of an energy source probably enhanced energy-dependent excretion of SON metabolites. Factors controlling SON production by heterogeneous activated-sludge cultures seem to be complex and interrelated, and definitive statements about the effect of individual factors is difficult. Nevertheless, the observed behavior remains; SON was produced in large quantities during culture start-up.

As MLSS concentrations increased to levels greater than 1700 mg/l, SON decreased, most likely due to utilization of produced SON by the increased concentration of organisms, and perhaps, due to decreased production and excretion of SON.

TABLE 58. THE EFFECT OF GROWTH RATE ON AS CULTURE 2 EFFLUENT SON DURING CULTURE START-UP[a]

Day of Operation	MLSS (mg/l)	Effluent SON (mg/l)	Effluent SCOD (mg/l)	μ (day^{-1})
7	232	2.47	43	0.14
10	412	1.99	40	
12	468	2.32	44	
13	680	2.40	44	
15	1050	2.87	49	
16	1220	2.11	42	
17	1350	1.83	44	
18	1480	1.26	38	
19	1630	1.02	31	
20	1730	0.94	29	
23	2100	1.43	49	
27	2010	2.11	60	-
steady-state[b]	2210	0.26	18	0.17

[a] Logarithmic growth occurred from Days 7 to 23 (see text).

[b] Average values for Days 104 to 176.

The second, smaller SON peak observed during AS Culture 2 start-up may have been a response to a temperature increase from the normal of 20°C to a maximum of 29°C on Day 27 because of an incubator failure. Culture color changed from a dark, golden brown to a much lighter brown between Days 23 and 30. Such color changes have been noted to signify subtle population shifts which affect effluent quality [217]. Other explanations are of course possible.

Steady-State Operation--

Steady-state operation was considered to be attained when MLSS concentration leveled off and remained relatively constant with time (± 250 mg/1). Table 59 contains a summary of steady-state data for periods meeting this criteria. AS Culture 1 initially reached steady-state operation after about 70 days (Figures 16 and 18), and AS Culture 2 after 80 days (Figure 17). Between Days 130 and 199, severe sludge bulking occurred in AS Culture 1 and several techniques (discussed later) were tried to alleviate the problem.

A t-test indicated the difference between the mean feed SON (0.10 ± 0.04 mg/1) and the mean culture SON values listed in Table 59 were statistically significant at the 99 percent level of confidence; this indicates SON was indeed produced during steady-state operation.

Effluent NH_3-N ranged from 0.2 to 19.8 mg/1 at steady-state operation for the two cultures, and nitrate-nitrogen never exceeded 0.41 mg/1, thus eliminating any concern over NO_3^--N interference with the Kjeldahl procedure for SON. Dissolved oxygen ranged between 5-6 mg/1 one hour after feeding to near 8 mg/1 at the end of the 23-hour aeration cycle. Routine microscopic observations of the cultures during steady-state operation showed predominance of large flocs of bacteria with free-swimming protozoa and an occasional rotifer. More

TABLE 59. SUM[illegible] OF STEADY-STATE DATA FOR ACTIVATED-SLUDGE CULTURES

Data Set Number	AS [illegible] Number: Days [illegible] Operation	SON*	SCOD*	MLSS*
1	1: Days 69-124	0.33 ± 0.04 (0.28-0.39) n = 14	12.0 ± 3.1 (7.2-19.4) n = 15	2060 ± 121 (1870-2300) n = 22
2	1: Days 204-293	0.63 ± 0.19 (0.40-1.11) n = 16	15.9 ± 5.0 (9.3-28.2) n = 16	2270 ± 153 (2040-2540) n = 15
3	2: Days 104-176	0.26 ± 0.05 (0.19-0.37) n = 14	18.1 ± 6.0 (7.7-33.6) n = 14	2210 ± 72 (2110-2330) n = 14

*All concentrations in mg/1; data are mean, standard deviation, and range (Brackets). n = number of samples analyzed.

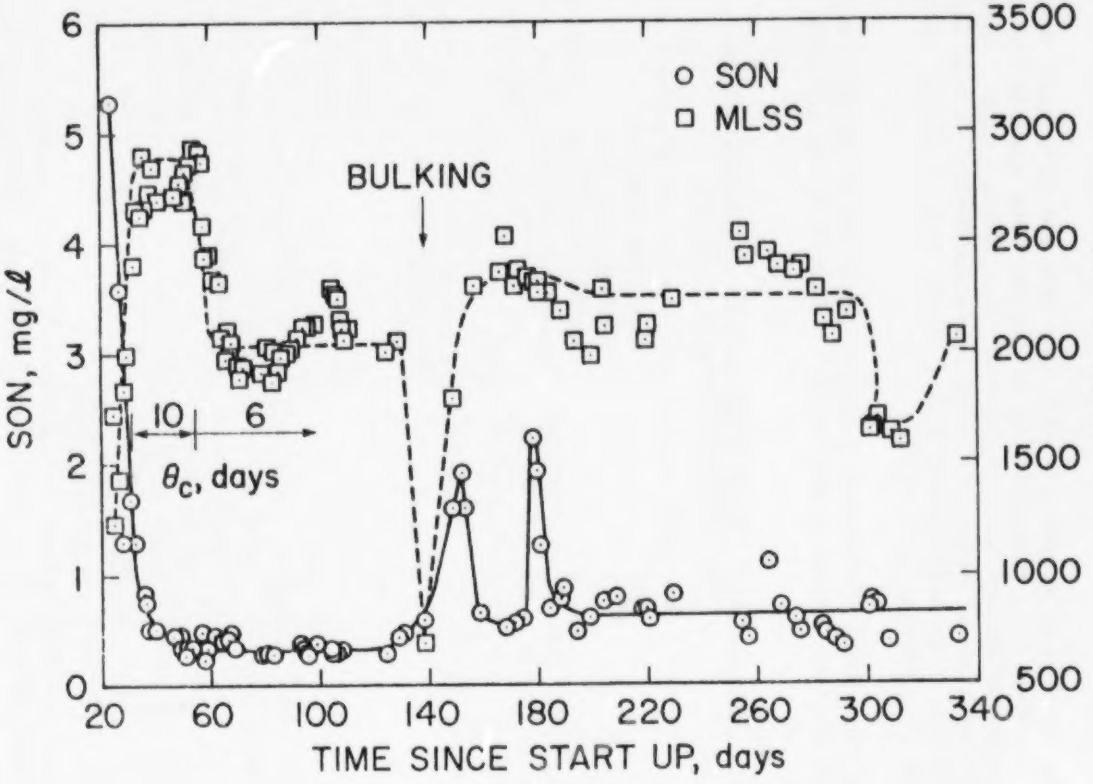


Figure 18. AS Culture 1, Days 20-340: SON and MLSS concentrations vs time of operation. Squares represent MLSS and circles represent SON.

filamentous organisms were observed during the period from Days 204-293 than the period from Days 69-124 for Culture 1. Culture color during steady-state periods ranged from light brown and grey to dark brown for AS Culture 1, and was predominantly light grey for AS Culture 2.

Effect of Population Changes--

Population shifts were suggested by obvious changes in culture color, settling characteristics, and microscopic examination (Table 60).

A t-test indicated that respective effluent SON and SCOD mean values for Data Sets 1 and 2 (Table 59), representing different time periods for Culture 1, were significantly different at a 99 percent confidence level. Thus, observed culture differences affected effluent characteristics, since all other experimental variables were held constant over those time periods. Other reports support this observation [207,217].

SON and SCOD data from AS Culture 2 (Data Set 3) were compared with Data Sets 1 and 2 (Seed Culture 1) using the t-test. Sample means differed

TABLE 60. CHARACTERISTICS OF MIXED LIQUOR SUSPENDED SOLIDS FOR ACTIVATED-SLUDGE CULTURES

Data Set Number	AS Culture Number: Days of Operation	Culture Color	Settling Characteristics of the Mixed Liquor Suspended Solids after 1 hour	General Microscopic Examinations
1	1: Days 69-124	Light brown	5 liters settled to about 500 ml	Many large bacterial flocs; free-swimming protozoa; some rotifers
2	1: Days 204-293	Dark brown to light brown to whitish grey to dark grey. Some foaming near end of period	5 liters settled to 200-900 ml	Some bacterial flocs; some filamentous forms present; few protozoa; no rotifers
3	2: Days 104-176	Light grey	5 liters settled to 500-800 ml	Large bacterial flocs; free-swimming protozoa; no rotifers

significantly at the 99 percent level for the SON data. SCOD means for Data Sets 1 and 3 were significantly different, while for Data Sets 2 and 3, they were not. Culture characteristics were different (Table 60) which may explain the differences in effluent SON and SCOD data.

Inspection of Figures 16, 17, and 18, and data listed in Table 59 show larger fluctuations in SON, SCOD, and MLSS for Data Set 2 than for Data Sets 1 and 3. In light of the previous discussion, and noting culture characteristics listed in Table 60, these larger fluctuations may be due to culture population shifts.

Data on the effect of population changes on SON production also emphasizes that growth rate did not control SON production. Growth rate was constant (0.17 day^{-1}) at all times (Tables 59 and 60), and yet significant changes in effluent SON levels occurred.

Sludge bulking was observed in AS Culture 1 approximately 130 days after initial start-up. After the daily 1-hour settling period the 5 liters of mixed liquor settled to levels ranging from 1.8 to 4 liters. Several potential remedies were tried including the addition of a Fe-Co solution. Effluent SON began to rise slightly as the degree of bulking increased. Commencing on Day 132, mixed liquor suspended solids which did not settle below the 1.8-liter level were wasted, resulting in the drop in MLSS noted in Figure 18. MLSS levels later began to increase until levels greater than 2000 mg/l were reached. An increase in SON was observed as MLSS increased rapidly, similar to that noted during culture start-up. SON then decreased rapidly as MLSS concentration leveled off. However, SON again rose sharply after the initial decrease as depicted in a second peak near Day 180 in Figure 18. No noticeable culture changes were observed during this period, and no suitable explanation for this second peak can be given. After the bulking problem, from Days 204 to 293, SON, SCOD, and MLSS varied more than during the period from Days 69 to 124, and were significantly different for the two periods as already noted.

Summary--

Activated-sludge cultures were developed to monitor SON production during start-up and during steady-state, semi-continuous operation. During the start-up phase, a maximum of 5 mg/l SON was produced. When cultures were operating at steady state, produced SON levels were 0.2-0.8 mg/l. Although growth rate and phase may affect the levels of SON produced by activated-sludge treatment, they were not the major factors involved here. Other factors such as starvation, substrate-accelerated death, energy-dependent excretion in response to the presence of a substrate, and bacterial utilization of produced SON were most likely involved. Significant differences in produced SON concentrations during steady-state operation were associated with different culture characteristics such as color, settling characteristics, and microscopic properties.

Initial SON Release

Objectives--

During batch removal experiments, SON was released from Palo Alto activated sludge upon dilution with tap water (see difference between calculated and observed time zero SON for controls, Figures 9 and 10). Subsequent dilution of activated-sludge solids resulted in release of additional SON. These data agree with observations that release of SON may be affected by concentration gradients [150,151,152]. A set of experiments was conducted to determine if this release of SON was real and reproducible, and what factors, if any, affected magnitude of the release.

Procedures--

Samples were taken within 30 seconds after dilution of culture with water and filtered as described under Batch Experiments to prevent further SON release or consumption. Laboratory AS cultures were used, experiments with Culture 1 being conducted 220 to 302 days after culture start-up, and with Culture 2, 124 to 173 days after culture start-up. The effects of MLSS concentration, culture source, temperature, and dilution water characteristics were studied.

The increase in SON (SON_q, mg/l) during those experiments was calculated as follows:

$$SON_q = SON_{om} - SON_{oc} \quad (8)$$

where SON_{om} is the initial measured SON (mg/l) and SON_{oc} is the calculated initial SON defined by Eq. 7 (mg/l). $SCOD_q$ is defined in a similar manner and is a measure of the additional SCOD released upon dilution of concentrated activated-sludge culture.

Effect of MLSS--

The effect of MLSS concentration on SON_q was evaluated using AS Culture 1. Experiments were conducted on 10 different days, and each day from one to five different MLSS concentrations were evaluated. Results are shown in Figures 19 and 20.

Average values of SON_q and $SCOD_q$ were 0.11 ± 0.04 and 4.0 ± 2.9 mg/l, respectively, and were both significantly greater than zero at the 99 percent level of confidence. Initially released SON and SCOD were independent of MLSS concentration, linear correlation coefficients (r) were only 0.02 and 0.37, respectively, for 23 MLSS concentrations ranging from 7 to 2390 mg/l.

The fact that initially released SON concentration was independent of MLSS concentration led to a hypothesis that certain nitrogen-containing organics diffuse through cell walls of microorganisms to establish an equilibrium concentration between the surrounding fluid and the microorganisms. This equilibrium concentration is independent of microorganism concentration. The hypothesis is consistent with discussion of the effect of concentration gradients [150,151,152] given previously.

Effect of Culture--

Initial SON release was evaluated for activated-sludge cultures from Palo Alto and from AS Cultures 1 and 2 as a function of MLSS concentration. Results are summarized in Table 61.

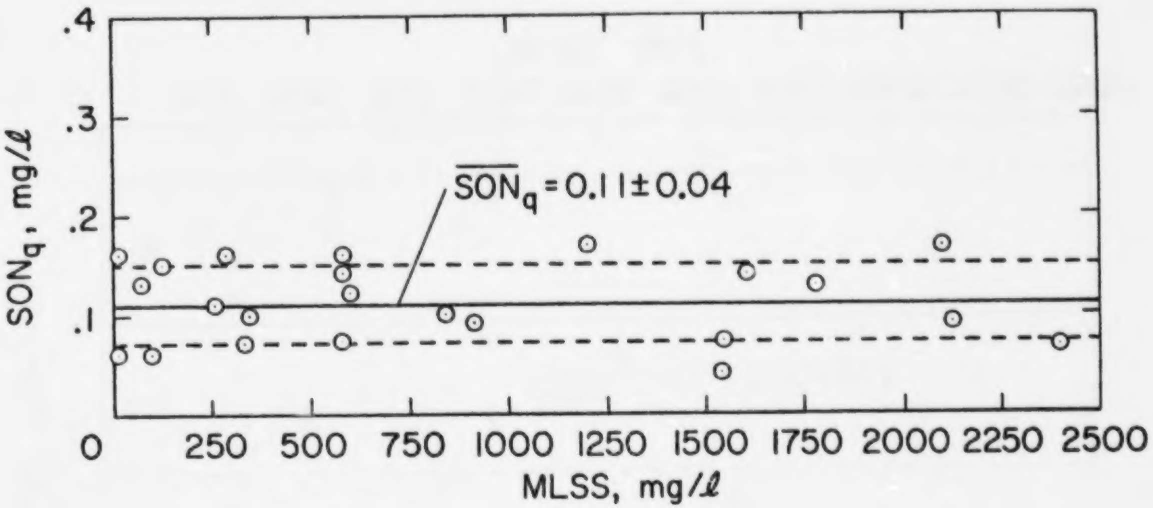


Figure 19. Initial release of SON for AS Culture 1 as a function of MLSS concentration.

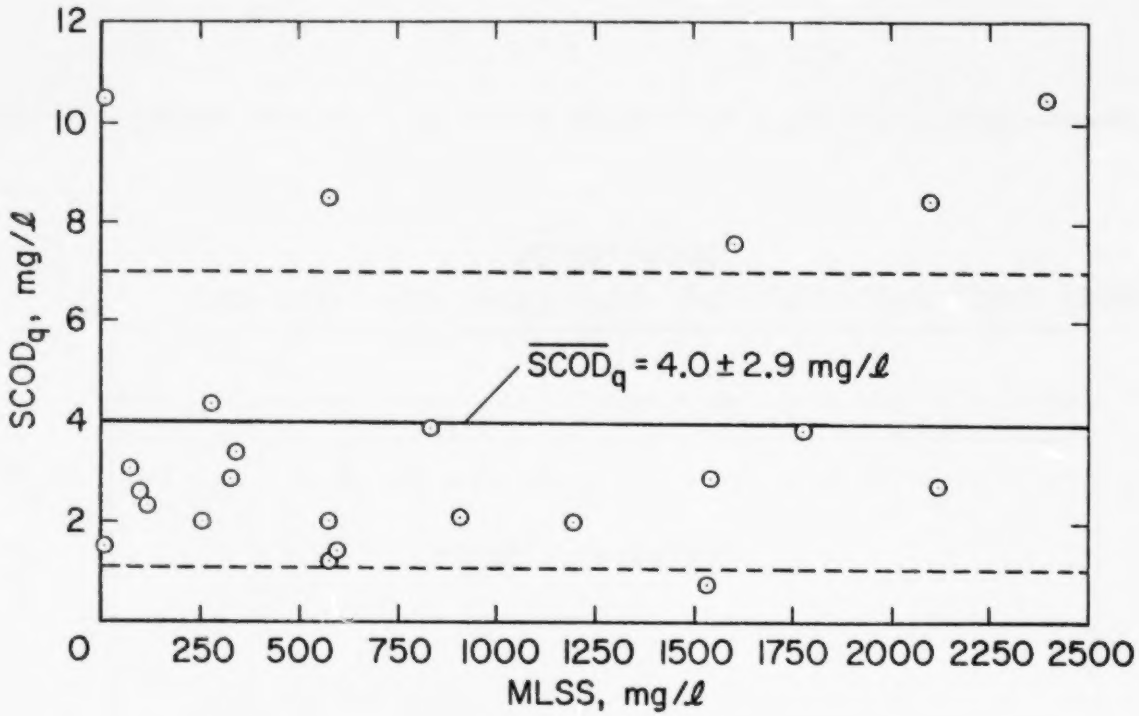


Figure 20. Initial release of SCOD for Seed Culture 1 as a function of MLSS concentration.

TABLE 61. EFFECT OF CULTURE ON $\underline{\mathrm{SON}}_q$ AND $\underline{\mathrm{SCOD}}_q$

Culture	MLSS Range (mg/l)	$\overline{\mathrm{SON}}_q$ (mg/l)	$\overline{\mathrm{SCOD}}_q$ (mg/l)	Number of sludge samples used	Number of MLSS values studied (n)
Palo Alto AS	2-1400	0.20 ± 0.09 (0.09)*	2.0 ± 2.0 (1.0)*	5	8
AS Culture 1	7-2390	0.11 ± 0.04 (0.02)*	4.0 ± 2.9 (0.6)*	10	23
AS Culture 2	120-1910	0.05 ± 0.01 (0.04)*	1.2 ± 0.4 (1.2)*	3	6

( )* $\overline{\mathrm{SON}}_q$ and $\overline{\mathrm{SCOD}}_q$ needed to be statistically greater than zero at a 95 percent level of confidence for n samples.

The individual SON means in Table 61 are significantly greater than zero at a 99 percent level of confidence, indicating that SON is indeed released when activated sludge is diluted. Release of SCOD was significantly greater than zero at a 99 percent confidence level for AS cultures, but only at a 95 percent level for Palo Alto AS.

Sample means were compared using the "one-factor analysis of variance" method described by Crow, Davis, and Maxfield [220], which states that differences in sample means (when comparing more than 2 means) are not considered significant unless those differences are large when compared with variations within samples. The method involves calculation of F values. Mean values of the three cultures for SON_q were significantly different at the 99.5 level of confidence, while mean SCOD_q values were significantly different at the 95 percent level. Thus, it can be concluded that the concentration of SON initially released is different for different cultures.

A second dilution of Palo Alto AS resulted in the release of additional SON (Table 62), providing support for the "equilibrium" level hypothesis. If the effect is truly an equilibrium effect, bacteria upon dilution should always excrete SON until an equilibrium concentration is reached, at least until the internal level of excretable SON drops below some critical level.

TABLE 62. $\underline{\mathrm{SON}}$ RELEASED BY SEQUENTIAL DILUTION OF PALO ALTO ACTIVATED SLUDGE

Dilution Number	SON_q (mg/l)	SCOD_q (mg/l)	MLSS (mg/l)
1	0.28	3.2	1400
2	0.45	3.1	1400

Testing the Equilibrium Hypothesis--

Three experiments were conducted to evaluate the equilibrium release hypothesis. In the first experiment, AS Culture 1 was added to both tap water and filtered AS Culture 1 supernatant. For the second and third experiments, AS Culture 1 was first added to tap water, the solids were settled, and the resulting supernatant liquor was filtered; then, additional AS Culture 1 was added. If the equilibrium hypothesis were correct, no additional SON should be released in the filtered supernatant samples, since they should already contain the equilibrium level SON. Results (Table 63) indicate that no additional SON was released as hypothesized. The values of SON_q and $SCOD_q$ for the controls are significantly greater than for the filtrate at a 95 percent level of confidence.

Effect of Temperature--

The effect of three different temperatures on initial SON release was evaluated. AS Culture 1 was equilibrated at the given temperature for at least one hour before use. Results in Table 64 indicate all values for SON_q and $SCOD_q$ were significantly greater than zero, but exhibited no dependence on temperature over the range studied as determined by correlation analysis.

Effect of Substrate--

The effect on initial SON release of mixing concentrated activated sludge with different substrates was evaluated using AS Culture 2. The substrates tested were as follows:

1. Tap water + $NaHCO_3$ (100 mg/l as $CaCO_3$, as for all previous studies),
2. Deionized water,
3. Tap water + $NaHCO_3$ + 15 mg/l NH_4Cl-N + 3 mg/l K_2HPO_4-P,
4. Tap water + $NaHCO_3$ + 15 mg/l NH_4Cl-N + 3 mg/l K_2HPO_4-P + 150 mg/l glucose-COD + 150 mg/l NaAcetate-COD, and
5. Seed Culture 2 filtrate.

All solutions were air saturated prior to culture addition. Results are presented in Table 65.

Although there were differences in SON_q for the various systems, the differences were not significant at a 95 percent level of confidence. Because of the low SON concentrations, analysis of several samples would be required to statistically evaluate whether the differences noted are significant. Additional information about the effect of substrate on the magnitude of SON_q is presented in the section on Significance of SON_q and $dSON_t$ Peaks.

Estimation of the True Equilibrium SON--

SON_q, the SON initially released upon dilution of activated sludge, is an apparent measure of an equilibrium SON concentration established between the organisms and their surrounding environment. The true equilibrium SON, termed SON_{eq}, will be the sum of SON_q plus the equilibrium SON remaining in the activated-sludge culture to be diluted, that is, a portion of SON_{oc} (Eq. 7).

TABLE 63. INITIAL RELEASE OF SON AND SCOD UPON EXPOSURE TO TAP WATER, AS CULTURE 1 FILTRATE, AND RELEASED ORGANICS

	Experiment 1 (255)[a] Culture SON = 0.56 mg/1, SCOD = 13.3 mg/1		Experiment 2 (256)[a] Culture SON = 0.43 mg/1, SCOD = 9.3 mg/1		Experiment 3 (302)[a] Culture SON = 0.79 mg/1, SCOD = 25.8 mg/1	
Substrate / Parameter[b]	Tap Water (Control)	AS Culture Filtrate	Tap Water (Control)	Filtered Organics Released from Control	Tap Water (Control)	Filtered Organics Released from Control
SON_{oc}	0.12	0.56	0.10	0.20	0.04	0.16
$SCOD_{oc}$	3.1	13.3	3.1	5.4	2.4	4.8
$MLSS_{om}$	1776	1776	904	904	252	330
SON_{om}	0.25	0.57	0.19	0.19	0.15	0.16
$SCOD_{om}$	6.9	12.9	5.2	4.8	4.4	6.4
SON_q	+ 0.13	+ 0.01	+ 0.09	- 0.01	+ 0.11	0
$SCOD_q$	+ 3.8	- 0.4	+ 2.1	- 0.6	+ 2.0	+ 1.6

[a] ( ) Day of AS Culture Operation.

[b] All in mg/1.

SON_q and $SCOD_q$ required to be significantly greater than zero at a 95 percent level of confidence are 0.06 and 1.7 mg/1, respectively, for n = 3.

TABLE 64. EFFECT OF TEMPERATURE ON INITIAL RELEASE OF SON AND SCOD[a]

Temp.	SON_q[b] (mg/l)	$SCOD_q$[c] (mg/l)	MLSS (mg/l)
10°C	+ 0.09	+ 2.6	570
20°C	+ 0.08	+ 2.2	520
30°C	+ 0.08	+ 2.6	560

[a] Seed Culture 1 sludge used; SON = 0.73 mg/l, SCOD = 20.1 mg/l.

[b] SON_q of 0.07 mg/l required for statistical significance at 95 percent confidence limit for n = 3.

[c] $SCOD_q$ of 1.7 mg/l required for statistical significance at 95 percent confidence limit for n = 3.

This sum can be written as

$$SON_{eq} = SON_q + (SON_{oc} - SON_{tap}) \left(\frac{SON_{eq}}{SON_e}\right) \tag{9}$$

Rearranging terms and solving for SON_{eq},

$$SON_{eq} = (SON_e) \left(\frac{SON_e}{SON_e - SON_{oc} + SON_{tap}}\right) \tag{10}$$

in which SON_e is the SON of the activated-sludge culture, SON_{oc} is the calculated initial SON, and SON_{tap} is the SON of the dilution water. A similar equation can be developed for $SCOD_{eq}$. Mean values calculated for SON_{eq} and $SCOD_{eq}$ are listed in Table 66.

All SON_{eq} and $SCOD_{eq}$ means were significantly greater than zero at a 99 percent level of confidence, and are independent of MLSS concentration (r values for SON_{eq} ranged from 0.05-0.24, and from 0.14-0.59 for SCOD). SON_{eq} and SON_q means (Table 61) are not significantly different for any of the cultures; the same was true for SCOD means.

Summary--

SON and SCOD are released upon dilution of activated sludge with tap water. The magnitude of the initially released SON is independent of MLSS concentration and temperature, but dependent on culture characteristics.

An equilibrium hypothesis was proposed, tested, and found to be consistent with the data and with reports by others [150,151,152]. Organisms

TABLE 65. INITIAL RELEASE OF SON AND SCOD: COMPARISON OF SUBSTRATES[a]

Parameter \ Substrate	Tap Water (Control)	Deionized Water	Tap Water plus Inorganics	Tap Water plus Inorganics plus Organics	AS Culture 2 Filtrate
MLSS (mg/1)	570	590	590	600	620
SON_{oc} (mg/1)	0.03	0.01	0.04	0.05	0.37
$SCOD_{oc}$ (mg/1)	3.1	1.0	3.1	270	23.8
SON_{om} (mg/1)	0.08	0.08	0.13	0.21	0.35
$SCOD_{om}$ (mg/1)	3.9	4.3	5.1	238	22.5
SON_q (mg/1)[b]	+ 0.05	+ 0.07	+ 0.09	+ 0.16	- 0.02
$SCOD_q$ (mg/1)[c]	+ 0.8	+ 3.3	+ 2.0	-32.1	- 1.3

[a] AS Culture 2; SON = 0.37 mg/1, SCOD = 24 mg/1.

[b] ± 0.10 mg/1 change required for 95 percent confidence limit (n = 1).

[c] ± 2.8 mg/1 change required for 95 percent confidence limit (n = 1).

[d] Definitions given in text and in Appendix A.

TABLE 66. EQUILIBRIUM SON AND SCOD VALUES FOR THE CULTURES STUDIES

Culture	MLSS Range (mg/l)	$\overline{\text{SON}}_{eq}$ (mg/l)	$\overline{\text{SCOD}}_{eq}$ (mg/l)
Palo Alto AS	2 - 1400	0.22 ± 0.10 (n = 8)	2.3 ± 2.5 (n = 8)
AS Culture 1	7 - 2390	0.12 ± 0.04 (n = 23)	4.5 ± 3.6 (n = 23)
AS Culture 2	120 - 1910	0.06 ± 0.01 (n = 6)	1.3 ± 0.4 (n = 6)

excrete certain SON compounds in order to reach an equilibrium concentration with these particular compounds. These compounds may be different from the majority of organics comprising effluent SON, since effluent SON concentrations are generally much higher. Regardless, "equilibrium" SON may comprise a significant portion of the SON produced during treatment. Mean SON_{eq} values for the three sludges examined were 0.22, 0.12, and 0.06 mg/l; corresponding mean SCOD_{eq} values were 2.3, 4.5, and 1.3 mg/l.

SON Release during Organism Decay

Objective--

SON released during organism decay (starvation conditions) is one possible source of activated-sludge effluent SON (see SON Production and Excretion by Bacteria). During initial studies on SON removal, significant quantities of SON and SCOD were released in tap water control systems, presumably due mostly to organism decay. The objective of the following studies was to better define the magnitude of the release during decay (SON_d and SCOD_d), and to evaluate factors affecting this release.

Procedures--

Organism decay during starvation was studied by aerating activated-sludge cultures in tap water. Data from previous batch studies using Palo Alto activated sludge were compared with similar batch studies using AS Cultures 1 from Days 268 to 293 and AS Culture 2 from Days 124 and 173. Tap water with $NaHCO_3$ (100 mg/l as $CaCO_3$) was aerated with an air-CO_2 mixture for 15-30 minutes prior to culture addition. Variables investigated were aeration time, and MLSS concentration.

Effect of Aeration Time--

Figures 21, 22, and 23 illustrate the effect of aeration time on SON and SCOD levels for concentrated Palo Alto AS (Batch Study 3) and AS Culture 1. Additional data on tap water systems for Palo Alto AS are found in Figures 9, 10, 14, and 15.

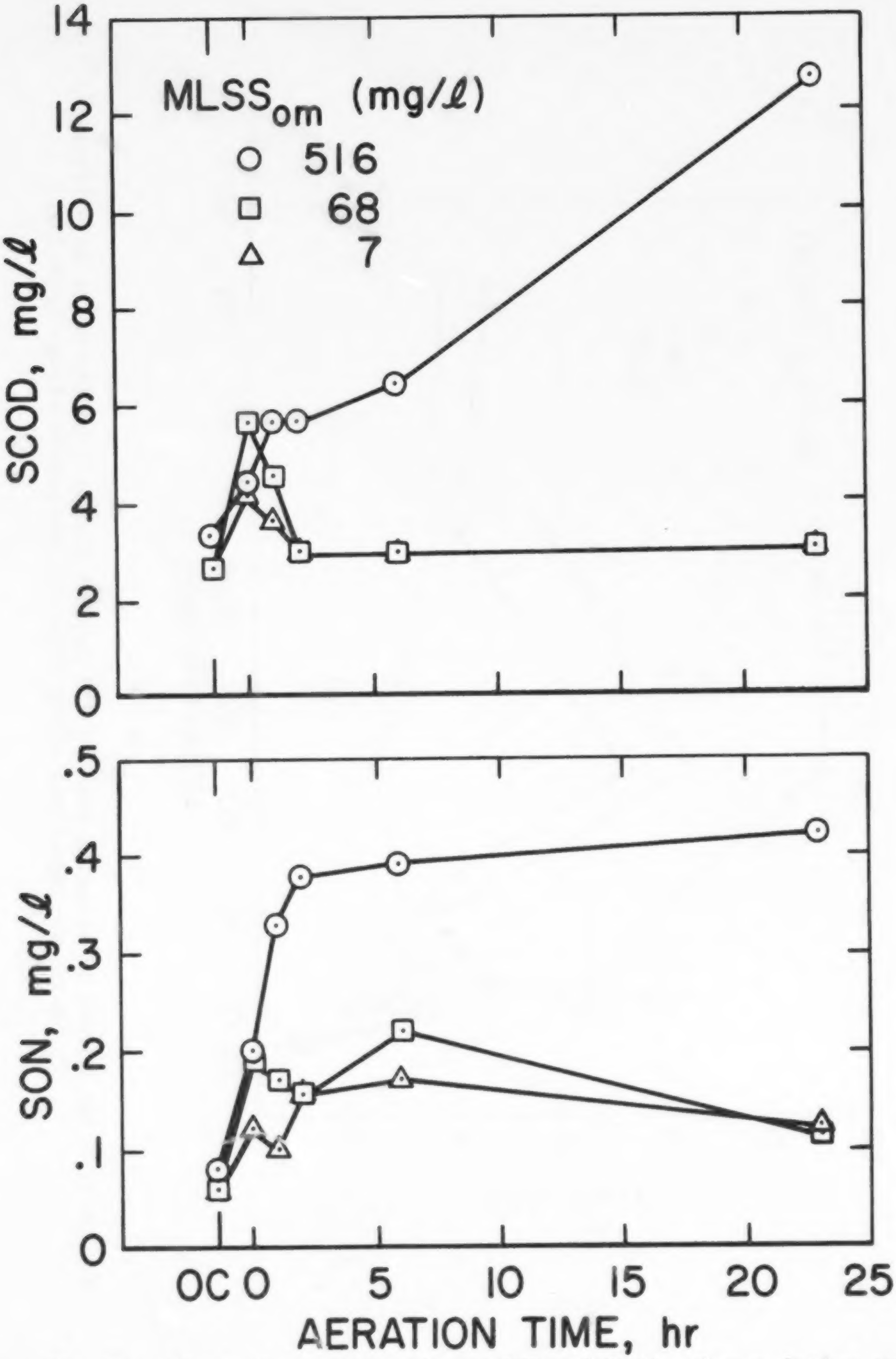


Figure 21. The effect of aeration time on SON and SCOD release during organism decay at different MLSS values using AS Culture 1 (Day 274).

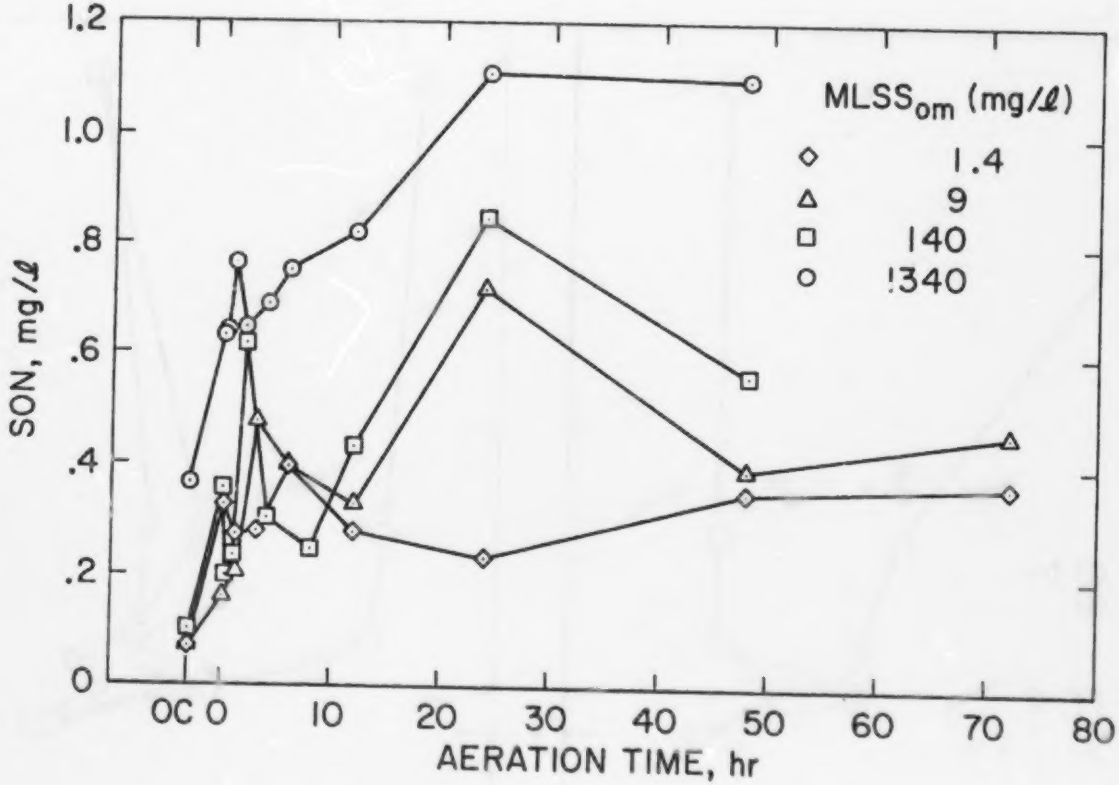


Figure 22. The effect of aeration time on SON release during organism decay at different MLSS concentrations using Palo Alto activated sludge (9-9-75).

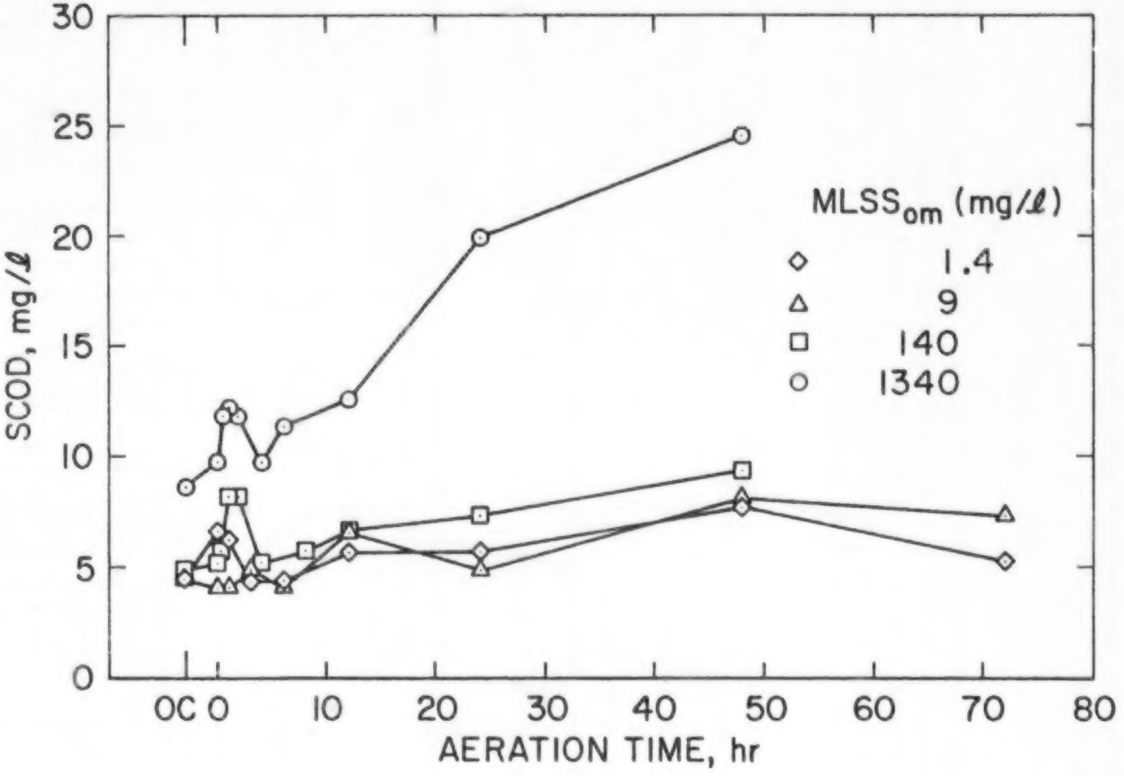


Figure 23. The effect of aeration time on SCOD release during organism decay at different MLSS concentrations using Palo Alto activated sludge (9-9-75).

SON released during aeration ($dSON_t$) is defined as

$$dSON_t = SON_{tm} - SON_{oc} \tag{11}$$

where SON_{tm} is the measured SON (mg/l) at aeration time t (hours), and SON_{oc} is the calculated initial SON (mg/l). Table 67 lists coefficients (a, b) obtained from regression analysis of data from Figures 21, 22, and 23 using the following equation:

$$dSON_t = a + bt \tag{12}$$

Similar expressions for $dSCOD_t$ were evaluated. SON released during organism decay was significantly correlated to aeration time at higher MLSS levels where analytical errors were not predominant. A similar observation was made for dSCOD. Peak concentrations in SON were noted to occur within the first few hours of aeration. This effect is explored in more detail in a later section.

The effect of long aeration periods on SON and SCOD released by organism decay was studied using aerobic digestors (see Batch Experiments). Two systems were operated. One with an "infinite" θ_c had increases from initial SON and SCOD values of 0.36 and 10.6 mg/l, respectively, to 1.11 and 17.3 mg/l. Nitrate nitrogen after 59 days was less than 10 mg/l, sufficiently low to avoid interference with SON analysis. During this period MLSS decreased from 2700 to 2000 mg/l and total organic nitrogen (TON) dropped from 242 to 196 mg/l. Since the increase in SON was only 0.75 mg/l (1.11 - 0.36), at least 98 percent of the 46 mg/l decrease in particulate TON was decomposed to

TABLE 67. SUMMARY OF REGRESSION ANALYSIS COEFFICIENTS CORRELATING SON AND SCOD RELEASE WITH AERATION TIME

Sludge Source	MLSS	$dSON_t$*			$dSCOD_t$†		
		a	b	Correlation Coefficient (r)	a'	b'	Correlation Coefficient (r')
AS Culture 1	516	0.23	0.01	0.63	1.5	0.33	0.99
"	68	0.13	- 0.003	0.66	1.7	- 0.07	0.54
"	7	0.08	- 0.001	0.21	0.9	- 0.03	0.55
Palo Alto AS	1340	0.31	0.01	0.90	1.8	0.31	0.96
"	140	0.23	0.01	0.57	1.3	0.06	0.63
"	9	0.27	0.002	0.37	- 0.03	0.05	0.82
"	1.4	0.23	0.001	0.35	1.1	0.01	0.26

* $dSON_t = a + bt$.

† $dSCOD_t = a' + b't$.

inorganic nitrogen. After 31 days of operating the 20-day θ_c system, SON and SCOD increased from 0.33 to 0.60 mg/l and 11.3 to 31.5 mg/l, respectively, MLSS and TON decreased from 2200 to 1700 mg/l and 190 to 123 mg/l, respectively, and NO_3^--N remained at zero. For this system, over 99 percent of the particulate TON lost was degraded to NH_3. Long periods of aeration do not result in large concentrations of released SON; organisms efficiently oxidize most of the cellular nitrogen-containing organics lost during organism decay. The same was found for SCOD.

Effect of MLSS--

The effect at different AS Culture 1 MLSS levels on the quantity of SON released during organism decay was evaluated by measuring the change in SON concentration over 23 hours of aeration, $dSON_{23}$, determined as follows:

$$dSON_{23} = SON_{23m} - SON_{oc} \tag{13}$$

SON_{23m} is the SON (mg/l) measured after 23 hours of aeration and SON_{oc} is the calculated initial SON concentration. Calculations for $dSCOD_{23}$ were made in a similar way. Experiments were conducted on four different days (between Days 283 and 293), and from two to four MLSS values were tested each time. Results are presented in Figure 24.

Coefficients calculated from linear regression analyses using the following equations are listed in Figure 24:

$$dSON_{23} = a + b\ MLSS \tag{14}$$

$$dSCOD_{23} = a_1 + b_1\ MLSS \tag{15}$$

Correlation coefficients of 0.96 and 0.86 were obtained for Eqs. 14 and 15, respectively, both indicating significant correlation of SON and SCOD release with MLSS of a 99 percent level of confidence (method of Crow et al. [220].) Standard errors of estimate for the two equations were 0.02 mg/l and 0.7 mg/l, respectively [218]. Values for SON_{eq} and $SCOD_{eq}$ for AS Culture 1 during these experiments were 0.09 ± 0.03 mg/l and 4.0 ± 2.0 mg/l, respectively.

These data support the equilibrium level hypothesis proposed earlier. If there is an equilibrium concentration of SON excreted by organisms, this material should always be present and should not be degraded during organism decay, i.e., $dSON_{23}$ should not decrease below SON_{eq} for any MLSS concentration. Extrapolation of linear regression equations to MLSS = 0 should yield SON_{eq} and $SCOD_{eq}$. Although SON_{eq} was somewhat larger than the MLSS = 0 intercept (a), SON_{eq} and a were not significantly different, implying that $dSON_{23}$ did not decrease below SON_{eq}. Similar observations were made about $dSCOD_{23}$; however the considerable scatter in the $SCOD_{eq}$ data makes detection of significant differences difficult.

Regression analyses of five MLSS concentrations (range: 110 - 1910 mg/l) of AS Culture 2 gave the following equations with significant correlation coefficients of 0.94 and 0.91, respectively:

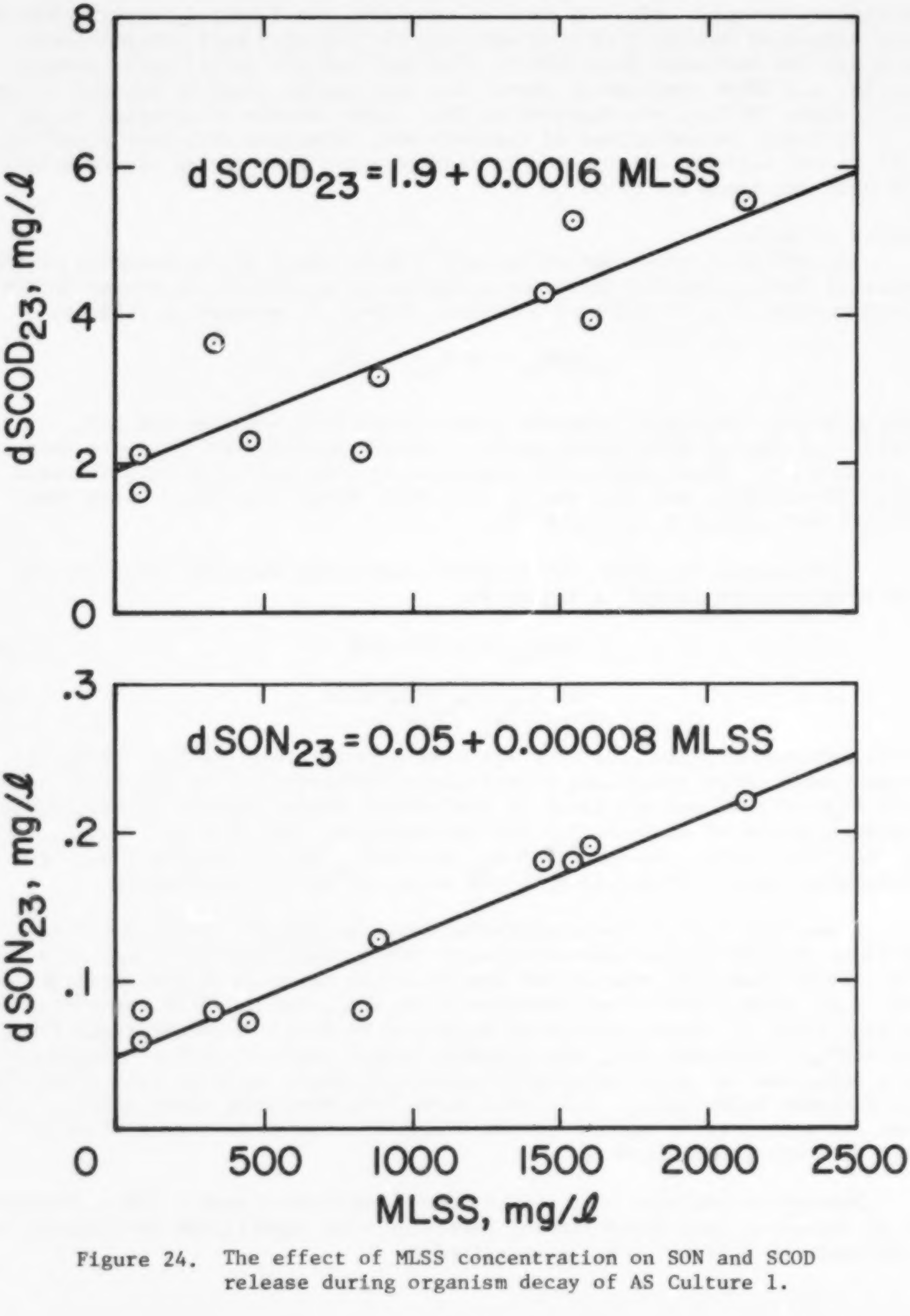


Figure 24. The effect of MLSS concentration on SON and SCOD release during organism decay of AS Culture 1.

$$dSON_{23} = 0.02 + 0.00008\ MLSS$$

$$dSCOD_{23} = 0.9 + 0.0021\ MLSS$$

Standard errors of estimate were 0.02 and 0.7 mg/l, respectively, SON_{eq} and $SCOD_{eq}$ were 0.06 ± 0.02 and 1.3 ± 0.4 mg/l, respectively, values not significantly different than $dSON_{23}$ and $dSCOD_{23}$, for MLSS = 0. These data provide additional support for the equilibrium hypothesis, and for the dependence of SON release during organism decay on MLSS concentration.

Additional data about the effect of MLSS can be obtained from experiments with Palo Alto Activated Sludge (Figures 22 and 23). Linear regression analysis was used to correlate $dSON_t$ (Eq. 11) and $dSCOD_t$ with MLSS. Aeration times of 12, 24, and 48 hours were selected for correlation of $dSON_t$ and $dSCOD_t$ since they should give representative values of the SON and SCOD released during organism decay.

Correlation coefficients listed in Table 68 show that SON released during organism decay is significantly correlated to MLSS concentration (Crow et al. [220]), except for the 24 hours aeration case; here, analytical error may have hindered the correlation. SCOD released during organism decay was most strongly correlated to MLSS, correlation coefficients were all in excess of 0.96. SON_{eq} and $SCOD_{eq}$ (0.22 ± 0.10 mg/l and 1.0 ± 1.1 mg/l, respectively) were not significantly different (95 percent confidence level) from $dSON_t$ and $dSCOD_t$ values for MLSS = 0 (Table 68), providing additional support for the equilibrium hypothesis. SON and SCOD concentrations were equal to or in excess of SON_{eq} abd $SCOD_{eq}$ under all aeration conditions, regardless of MLSS concentration.

TABLE 68. LINEAR REGRESSION EQUATIONS SHOWING THE EFFECT OF MLSS ON SON AND SCOD RELEASE DURING ORGANISM DECAY OF PALO ALTO ACTIVATED SLUDGE

$dSON_{12} = 0.26 + 0.00015\ MLSS$	(r = 0.92, e = 0.04)
$dSON_{24} = 0.50 + 0.00020\ MLSS$	(r = 0.45, e = 0.13)
$dSON_{48} = 0.34 + 0.00030\ MLSS$	(r = 0.95, e = 0.05)
$dSCOD_{12} = 1.6 + 0.00169\ MLSS$	(r = 0.96, e = 0.4)
$dSCOD_{24} = 1.0 + 0.00764\ MLSS$	(r = 1.00, e = 0.4)
$dSCOD_{48} = 3.4 + 0.00934\ MLSS$	(r = 1.00, e = 0.3)

r = correlation coefficient, e = standard error of estimate.

MLSS values tried were 1340, 140, 9, and 1.4 mg/l.

Summary--

Experiments were conducted to study the release of SON and SCOD during organism decay. Most of the cellular organic nitrogen and COD lost during decay is converted to inorganic end products; only a small percentage accumulates in the surrounding fluid as SON and SCOD. The SON and SCOD produced during organism decay is strongly correlated with MLSS, increased SON and SCOD concentrations are associated with higher MLSS concentration. Extrapolation of linear regression equations for released SON and SCOD vs MLSS to MLSS = 0 resulted in residual SON and SCOD concentrations equivalent to values found for SON_{eq} and $SCOD_{eq}$, lending further support to the equilibrium hypothesis.

SON Release with Synthetic Feed

Objectives--

The objectives of these studies were to better define SON_p and SON_g (the SON produced specifically as a result of substrate oxidation) by determining the effect of initial SCOD, MLSS, and NH3-N, and substrate type on production of SON. Substrates were chosen which contained little SON, and laboratory AS Cultures were used since they were grown on substrates with little SON.

Procedures--

Batch studies using synthetic feed and AS Cultures 1 and 2 were conducted. For some studies quantities of culture were required that were greater than the one liter available from routine wasting. Increased culture harvest was accomplished by not wasting on days prior to experiments, and adjusting feed volumes, waste supernatant volumes, and mass of COD fed to maintain a constant growth rate. In this manner the required quantity of sludge was produced without changing seed culture growth characteristics. Table 69 lists variables studied, and filtration and SON techniques used.

Batch Studies 6 and 7 were conducted with AS Culture 1 from Days 61 and 98, respectively, after start-up, and the other studies were conducted with AS Culture 2, 140 and 180 days after start-up. Culture growth was stable during these periods. Dissolved oxygen was greater than 1.5 mg/l, and pH was between 7.0 and 7.5 during all experiments. During Batch Studies 6 and 7,

TABLE 69. VARIABLES STUDIED AND FILTRATION AND SON TECHNIQUES USED DURING SYNTHETIC FEED STUDIES

Batch Study No.	Variable	Filtration Technique*	SON Analytical Technique
5	SCOD	Glass Fiber - Pall Filtration	Kjeldahl
6	SCOD	Centrifugation - Syringe Millipore	Technicon
7	Substrate Type	Centrifugation - Syringe Millipore	Technicon
8	MLSS	Glass Fiber - Pall Filtration	Kjeldahl
9	NH_3-N	Glass Fiber - Pall Filtration	Kjeldahl

*As described in Chapter 4.

NH_3-N was maintained above 5 mg/l so that nitrogen would not be growth-limiting, and below 20 mg/l to minimize analytical problems in the Technicon procedure.

Values of $dSON_t$ (Eq. 11) vs aeration time were calculated since they should give the most reasonable estimate of the SON produced in excess of that present in the feed solution and seed sludge at the beginning of the experiment. Use of $dSON_t$ also helps correct for possible NH_3-N carry-over during SON analysis by the Technicon method.

Statistical Analysis--

Values for SON obtained during these studies were low, necessitating statistical analysis for data evaluation. Answers to two questions were sought: (1) Was SON produced during the treatment, and (2) Did the variable studied affect SON production?

The first question was answered using the following form of Eq. 11:

$$dSON_t(i) = SON_{tm}(i) - SON_{oc}(i) \qquad (16)$$

where $SON_{tm}(i)$ is the SON concentration measured at time t under test condition i, and $SON_{oc}(i)$ is the calculated initial SON for test condition i. Values of $dSON_t(i)$ for all aeration times, including time 0, were combined using Eq. 17 to yield a mean value and standard deviation (n samples) for each test condition in a given study (i.e., one for SCOD = 1000, one for SCOD = 500, etc.):

$$\overline{dSON_t(i)} = \left(\sum_{t=0} dSON_t(i)\right)/n \qquad (17)$$

A t-test was used to determine if the mean was significantly greater than zero at a 95 percent level of confidence.

For the second question, the effect of aeration time was first ascertained by a linear regression analysis of the following:

$$dSON_t(i) = a + bt \qquad (18)$$

If aeration time was not significantly correlated with $dSON_t$ (95 percent level of confidence as determined with the method described by Crow et al. [220]), question 2 posed above was answered using a linear correlation analysis of Eq. 19:

$$\overline{dSON_t(i)} = a + bC \qquad (19)$$

in which $\overline{dSON_t(i)}$ is defined by Eq. 17, and C is the concentration (mg/l) of the variable being tested. The strength of this correlation was measured to a correlation coefficient, a 95 percent or higher level of confidence was considered necessary for the correlation to be significant [220]. A second method employed to answer question 2 involved comparing experimental test conditions using the following equations:

$$dSON_t(i-j) = dSON_t(i) - dSON_t(j) \tag{20}$$

$$\overline{dSON_t(i-j)} = \left(\sum_{t=0} dSON_t(i-j)\right)/n \tag{21}$$

where $dSON_t(i-j)$ is the difference in $dSON_t$ values (Eq. 11) for test condition i and control condition j, $\overline{dSON_t(i-j)}$ is the mean difference averaged over all aeration times, and n is the number of samples analyzed. A t-test indicated whether $\overline{dSON_t(i-j)}$ differed significantly from zero at a 95 percent level of confidence. This method was used to evaluate data from Batch Studies 6 and 7 in which SON varied significantly with aeration time, but not in a linear fashion.

Effect of Initial SCOD--

In Batch Studies 5 and 6 the effect of $SCOD_{oc}$ (calculated initial SCOD) on SON production was evaluated using AS Cultures 2 and 1, respectively. Feed solutions contained equal concentrations of glucose and acetate COD. A tap water control was used during Batch Study 6 but not during Batch Study 5. Initial SCOD:N ratios were 25:1 for Batch Study 5. Ammonia-N was added in steps (described earlier) to the 954- and 477-mg/l systems of Batch Study 6. The ratio of added N:P was 5:1 for all systems. Results are presented in Figures 25, 26, and 27.

SON was produced during oxidation of the synthetic substrates at observed levels of significance of 98 percent for Batch Study 5 and 99 percent for Batch Study 6, but $dSON_t$ was not significantly correlated with aeration time for either batch study. There was no significant correlation of $\overline{dSON_t}$ with $SCOD_{oc}$ for Batch Study 5; in other words, influent SCOD concentration could not be shown to affect the concentration of SON produced.

The concentration of SON released after 23 hours aeration with glucose-acetate was not statistically different from that released after 23 hours of aeration with no feed (organism decay for AS Culture 2). This observation was made by comparing results from Batch Study 5 with the following linear regression equation developed from decay studies with the same cultures and over the same time period (see preceding section):

$$dSON_{23} = 0.02 + 0.00008 \text{ MLSS}$$

An approximate standard error of estimate for the above equation is ± 0.02 mg/l as calculated by the method of Spiegel [218]. For an MLSS of 580 mg/l, the average initial MLSS concentration in Batch Study 5, a value of 0.07 ± 0.02 mg/l is predicted for the SON released during organism decay. Values of $dSON_{23}$ for the systems evaluated in Batch Study were 0.13, 0.08, 0.06, and 0.07 mg/l, which are not significantly different from the predicted value. Thus, the concentration of SON produced after 23 hours aeration in the presence of an exogenous carbon source and that produced after 23 hours of organism decay are apparently equal. The nature of the SON may be different, but concentrations are similar, and very low.

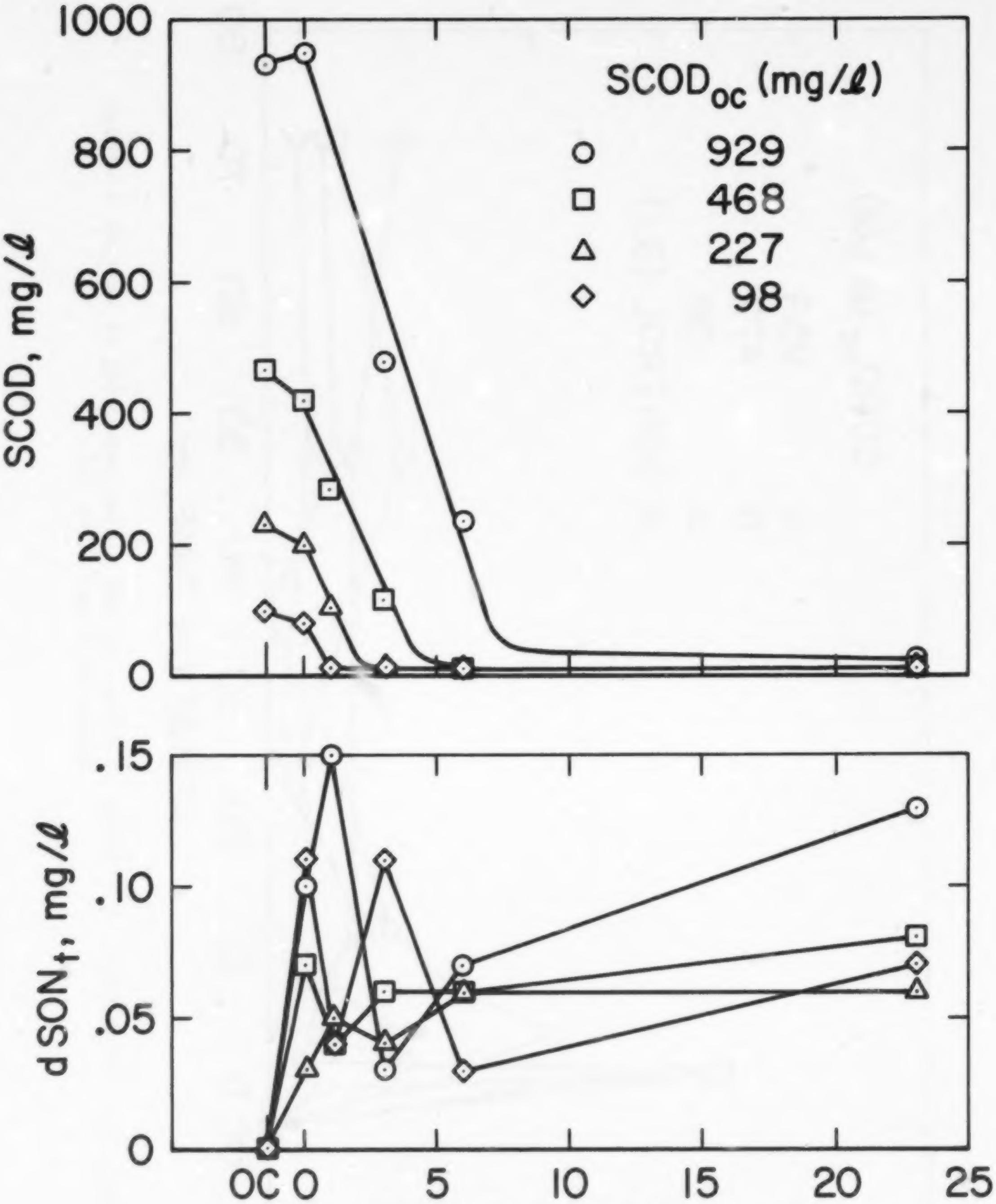


Figure 25. Batch Study 5: Effect of SCOD$_{oc}$ on SON production using AS Culture 2, MLSS$_{om}$ ≈ 580 mg/l (Day 171).

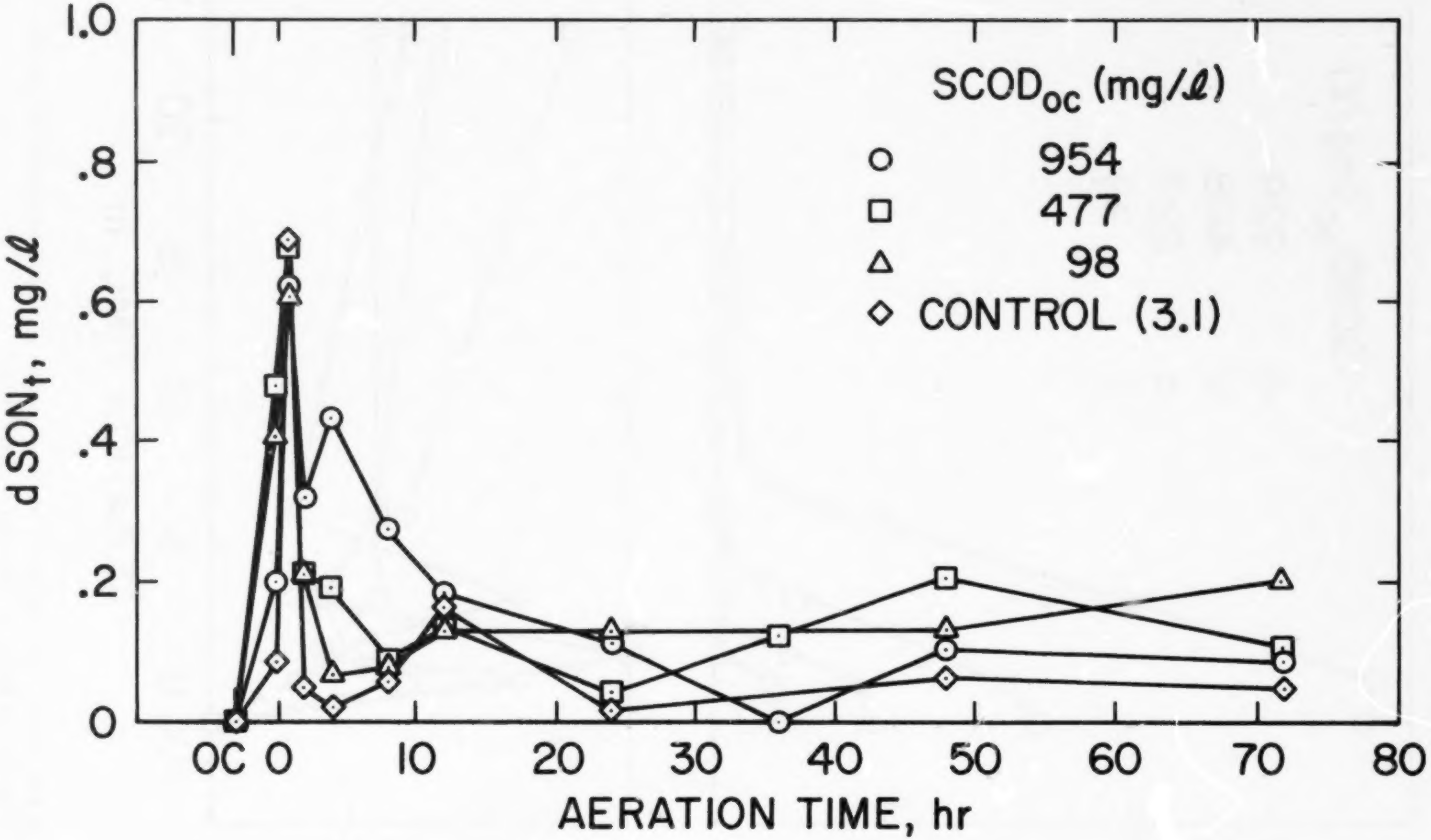


Figure 26. Batch Study 6: Effect of SCOD$_{oc}$ on SON production using AS Culture 1 sludge, MLSS$_{om}$ ≃216 mg/l (Day 61).

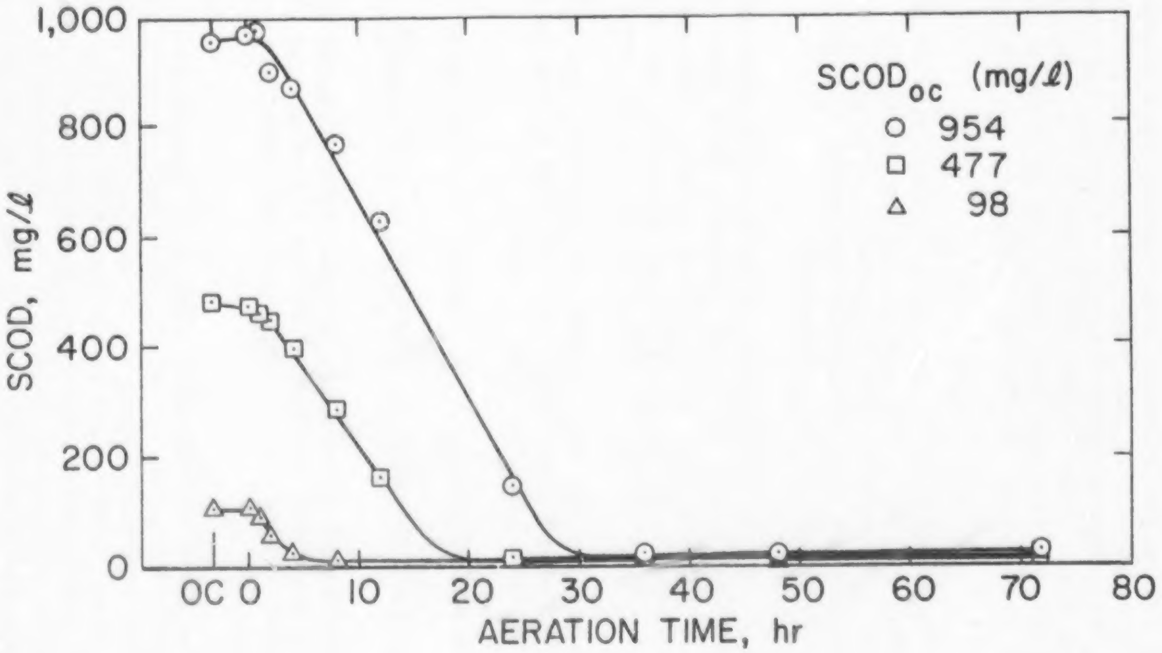


Figure 27. SCOD removal vs aeration time in Batch Study 6, $MLSS_{om} \simeq 216$ mg/1 (Day 61).

However, in Batch Study 6 using AS Culture 1, the presence of a utilizable substrate resulted in a significantly increased level of produced SON, as determined by a comparison of the three systems fed glucose-acetate with the tap control system using Eq. 21. This increase, an average of 0.10 ± 0.13 mg/l for the 27 samples analyzed, was significant at a 99 percent level of confidence.

Differences in SON production behavior have already been linked to differences in cultural characteristics, and these differences seem further exemplified by the results from Batch Studies 5 and 6. However, SON concentrations were so low during Batch Study 5 with AS Culture 2 that limitations in the sensitivity of the SON analysis may have prevented observance of substrate effects on SON and SCOD production. A similar limitation was not present with Batch Study 6.

Comparison of the SON production by the three fed systems for Batch Study 6 using the 98-mg/l $SCOD_{oc}$ system as the control condition (Eq. 21) indicated that the concentration of SON produced was independent of glucose-acetate $SCOD_{oc}$. Evidently, the presence of a utilizable substrate, but not its absolute concentration, was important in SON production.

Although there was no significant linear correlation between $dSON_t$ and aeration time, inspection of Figures 25 and 26 suggests there were significant differences in SON concentrations over the aeration period. The effect, a noticeable $dSON_t$ peak within the first two hours of aeration, was most pronounced in Batch Study 6. This effect is addressed in a later section.

SCOD removal was linear in the two studies, as expected, and minimum effluent SCOD was a function of influent SCOD, as indicated by the following correlations for Batch Studies 5 and 6, respectively:

$$dSCOD_{min}(5) = 0 + 0.0079\ SCOD_{oc}\ , \quad (r = 0.99, \quad e = 0.4\ mg/l)$$

$$dSCOD_{min}(6) = 1.1 + 0.0142\ SCOD_{oc}, \quad (r = 0.99, \quad e = 0.6\ mg/l)$$

where $dSCOD_{min}$ is the difference between the minimum SCOD measured during the experiment ($SCOD_{min}$) and the SCOD contributed by the tap water and AS seed. $SCOD_{oc}$ is the initially calculated SCOD. Similar linear dependency has been reported by others [50,62,214].

Extrapolation of the above equations to $SCOD_{oc} = 0$ should yield values approaching $SCOD_{eq}$ for the two cultures. $SCOD_{eq}$ for Batch Study 5 was 1.3 ± 0.4 mg/l, based on samples analyzed during the same time period, and $SCOD_{eq}$ for Batch Study 6 was 3.0 mg/l, the value calculated from the tap water control (Figure 27). These $SCOD_{eq}$ values are not significantly different from extrapolated values. While limitations in analytical technique at such low SCOD levels may have prevented significant differences from being observed, when viewed in combination with previous data regarding equilibrium SON and SCOD concentrations, data from Batch Studies 5 and 6 lend further support to the equilibrium hypothesis.

Effect of Substrate Type--

The objective of this experiment (Batch Study 7) was to determine if substrate type affects SON production. Solutions used were tap water and tap water with approximately 860 mg/l COD of glucose, acetate, and a glucose-acetate mixture. NH_3-N was added to maintain a range of 5-20 mg/l N, and added N:P ratio was 5:1. AS Culture 1 was used, and results are depicted on Figures 28 and 29.

Significant quantities of SON were produced by all systems, but there was no linear correlation between produced SON and aeration time. Also, no significant difference could be shown between the concentration of SON produced by the three substrate-fed systems and the control system.

Noticeable differences in $dSON_t$ over the aeration period were again observed, the effect being most dramatic within the first hour of aeration when large $dSON_t$ peaks were observed. This will be discussed later. SCOD removal was again linear.

Effect of MLSS--

In Batch Study 8 the effect of initial MLSS concentration ($MLSS_{om}$) was evaluated on three different occasions with AS Culture 2. $MLSS_{om}$ values ranged from 150 to 2000 mg/l, $SCOD_{oc}$ was kept relatively constant near 270 mg/l, and initial SCOD:N and N:P ratios were 25:1 and 5:1, respectively. Results are summarized in Figure 30.

SON production was statistically significant, although sometimes very low, was not correlated to aeration time, but was correlated linearly to $MLSS_{oc}$ ($r = 0.89$ [220]). Concentrations of produced SON appeared similar to those resulting from organism decay, and linear regression equations for aeration times of 23 hours indicated the differences were not statistically significant:

$$dSON_{23}\ (\text{organism decay}) = 0.02 + 0.00008\ MLSS$$

$$dSON_{23}\ (SCOD_{oc} \simeq 270\ \text{mg/l}) = -0.01 + 0.00009\ MLSS$$

AS Culture 2 data for the above equations were taken over the same time period (Days 156 to 173). Standard errors of estimate are 0.02 and 0.03 mg/l, respectively [218]. The observation here agrees with Batch Study 5: it is difficult to distinguish between SON produced in the presence of utilizable carbon and that produced during organism decay.

Effect of NH_3-N--

Batch Study 9 was conducted to evaluate whether initial NH_3-N concentration (NH_3-N_{oc}) affected SON production. Feed solution $SCOD_{oc}$ was maintained constant at around 500 mg/l, and NH_3-N was initially added in concentrations varying from 0 to 60 mg/l. Results are shown in Figure 31. SON production was statistically significant for all three systems, and was independent of aeration time and initial NH_3-N concentration.

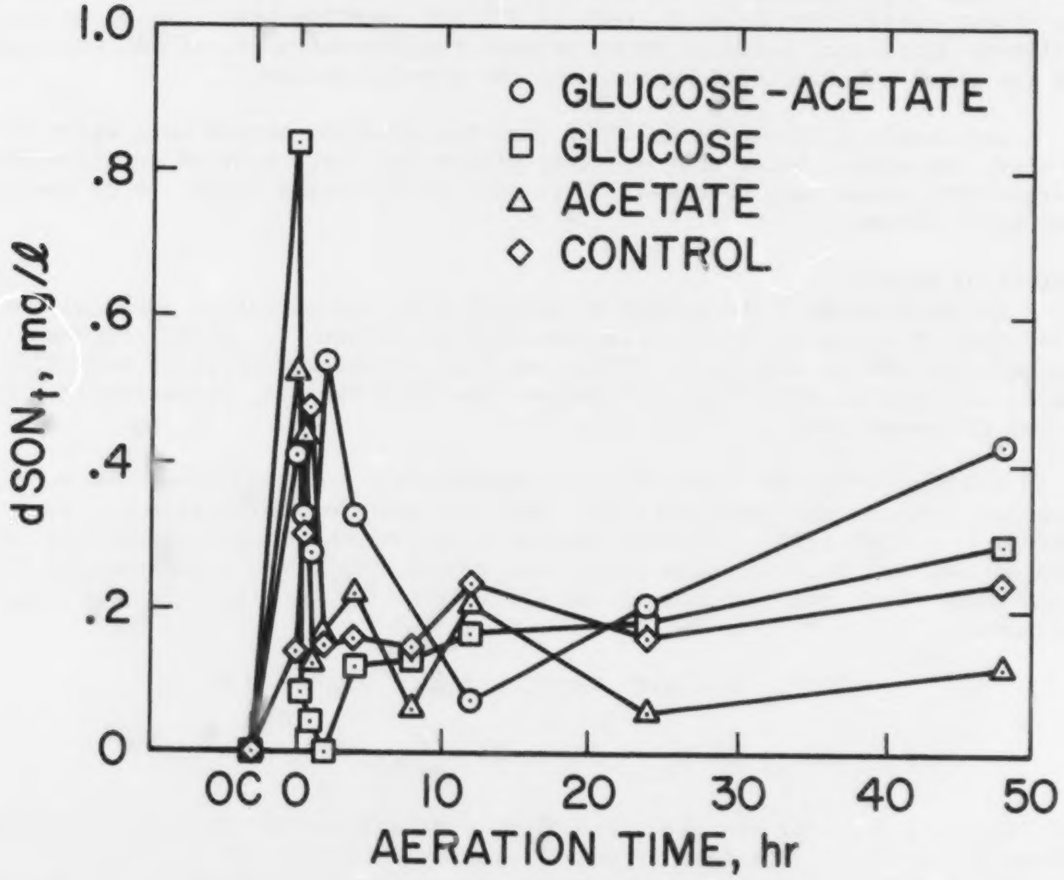


Figure 28. Batch Study 7: Effect of substrate type on SON production using AS Culture 1, $MLSS_{om}$ = 360 mg/l (Day 98).

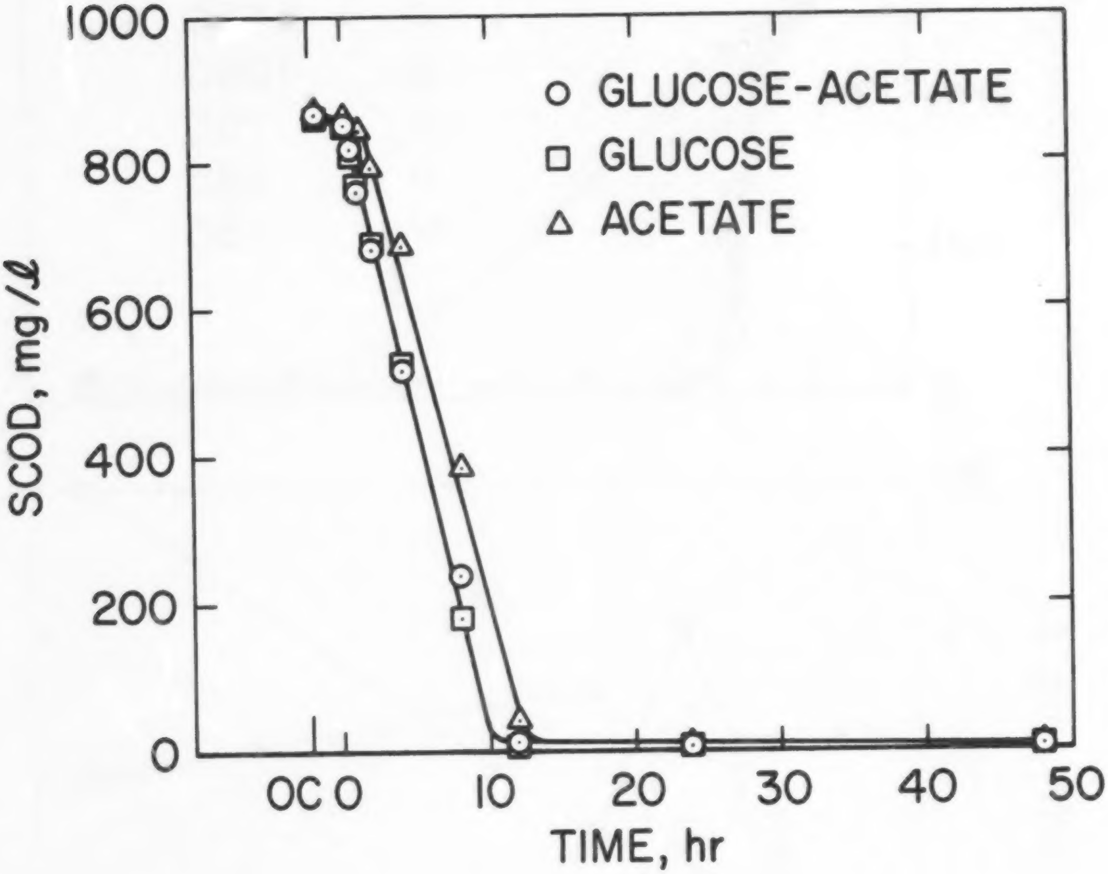


Figure 29. Batch Study 7: SCOD removal vs aeration time, $MLSS_{om} \simeq 360$ mg/l (Day 98).

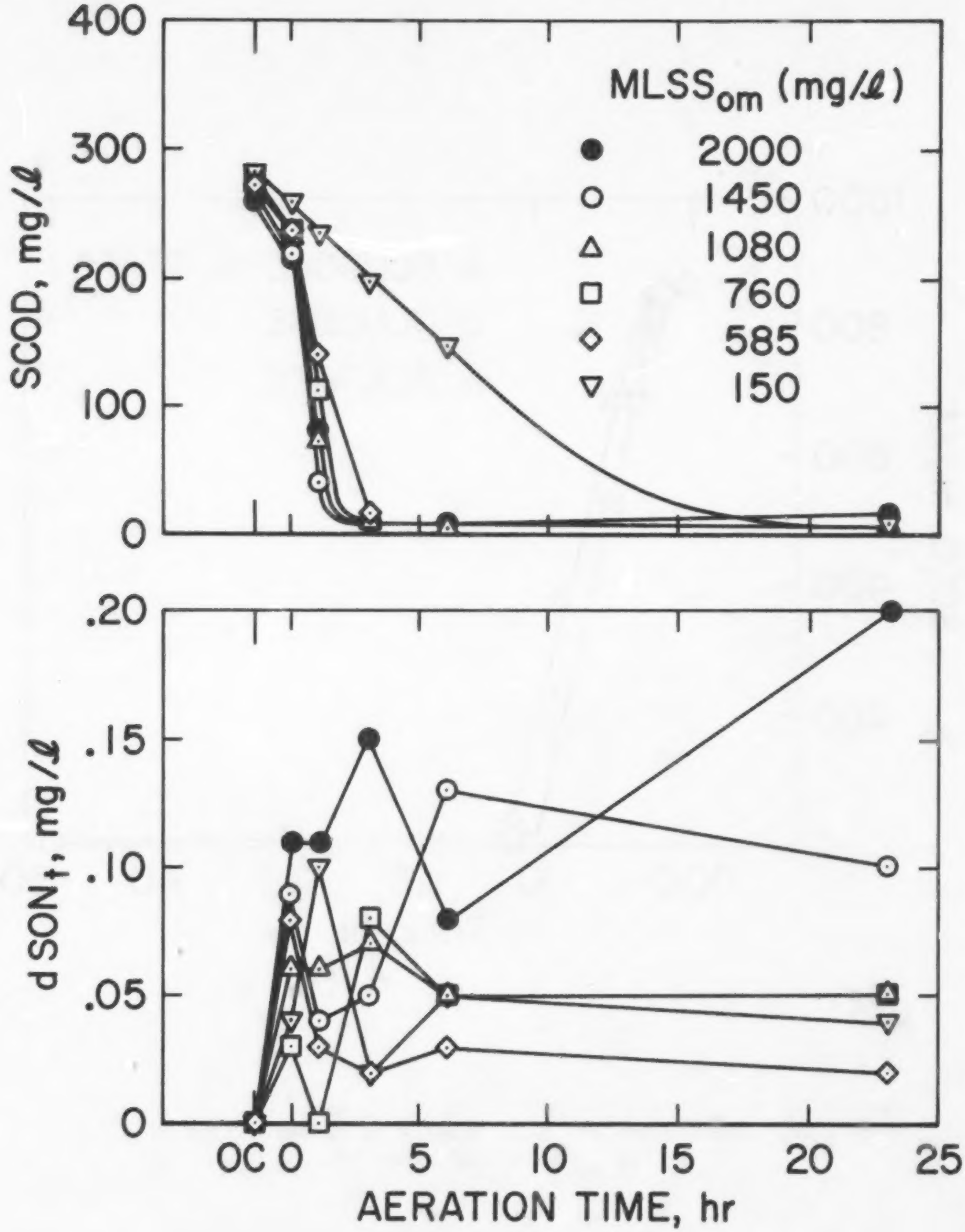


Figure 30. Batch Study 8: Effect of MLSS$_{om}$ on SON production.

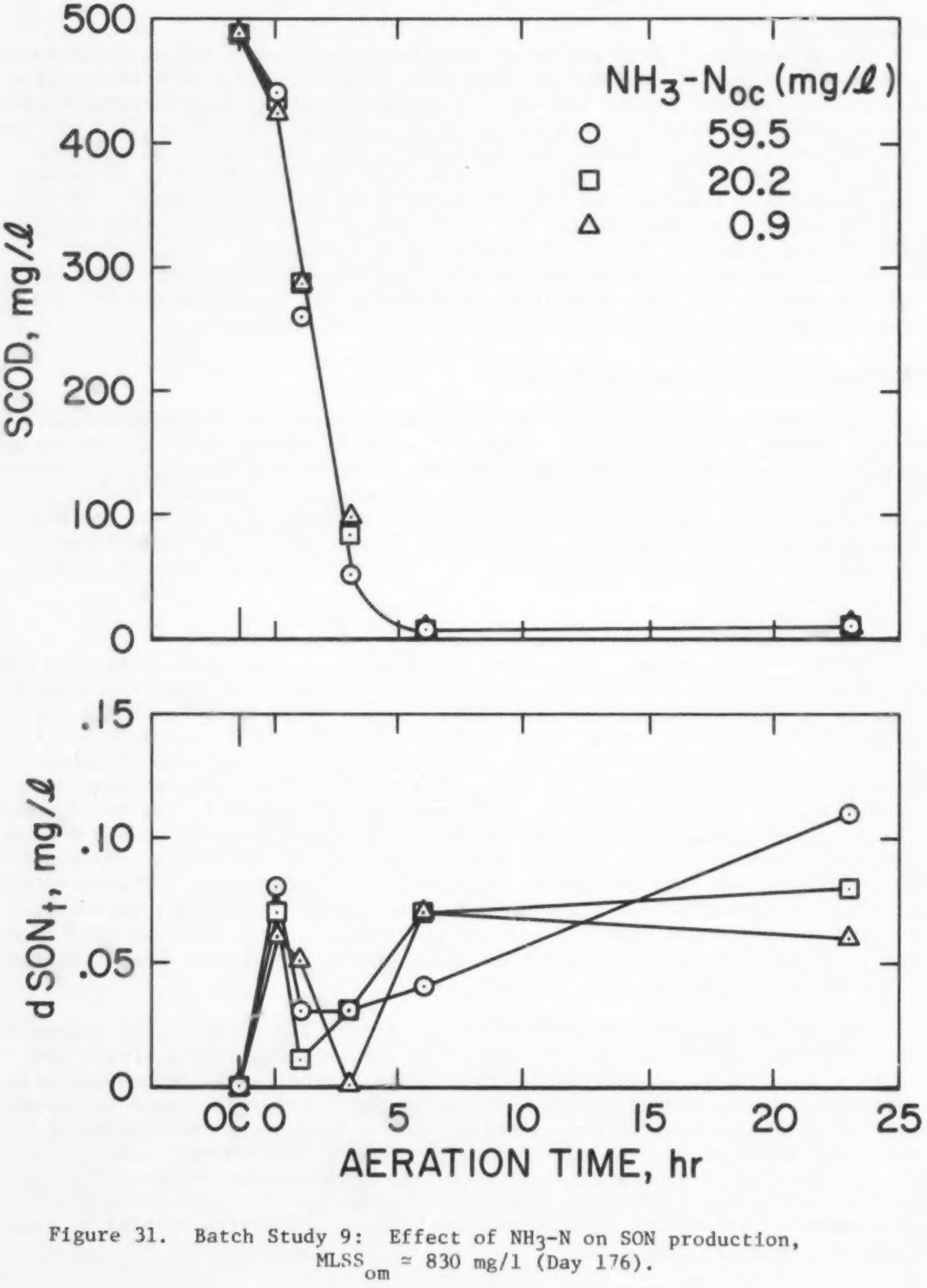


Figure 31. Batch Study 9: Effect of NH_3-N on SON production, $MLSS_{om} \approx 830$ mg/l (Day 176).

Summary--

The objective of this series of experiments was to determine which variables and factors affect SON_p and SON_g (SON produced as a result of the presence of a utilizable carbon source). Although one study showed statistically that more SON was produced by systems fed a utilizable substrate than by control systems (organism decay), the majority of the data indicated that SON levels produced by fed and control systems were not significantly different. This observation implies that SON_g is zero; or at least very small. SON was produced during all experiments, but only initial MLSS concentration could be shown to significantly affect the amount of SON produced. Initial SCOD, NH_3-N, and substrate type did not affect produced SON levels, but SON concentrations were so low that analytical limitations may have hindered the detection of significant differences, especially for AS Culture 2.

Significance of SON_q and $dSON_t$ Peaks

Two general types of SON peaks were noted during SON production experiments: initially released peaks (SON_q, defined by Eq. 8) and peaks occurring during aeration ($dSON_t$). The SON initially released upon dilution of culture with tap water (see Initial SON Release) appeared to establish a concentration which was in equilibrium with an organic nitrogen pool within the cells. This equilibrium concentration, SON_{eq}, was independent of MLSS concentration (10-2400 mg/l) and temperature (10-30°C), but dependent on cultural characteristics.

SON_q Peaks--

Table 70 is a summary of initial SON release for the various studies conducted and indicates that the presence of a utilizable substrate affected the quantity of initially released SON (SON_q). Mean values of SON_q ($\overline{SON_q}$) for systems fed utilizable carbon were 0.48 ± 0.17 mg/l (n = 7) and 0.07 ± 0.04 mg/l (n = 10) for AS Cultures 1 and 2, respectively. Comparison of these means with respective values for SON_{eq} (Table 70) showed a significant difference at a 99 percent level of confidence for AS Culture 1, but not for Culture 2. The presence of an exogenous substrate caused release of SON concentrations in excess of SON_{eq} for Culture 1, but not for Culture 2. However, SON concentrations were so low for Culture 2 that significant differences may not have been detectable. Both cultures released SON initially; Culture 1 released significantly more (99 percent confidence limit) SON than Culture 2. Others [207,217] have also shown that differences in cultural characteristics affect process performance.

The excess initial SON released by AS Culture 1 was due to the presence of a utilizable substrate and was therefore part of SON_g. This excess SON may be a function of growth rate or growth phase, may result from substrate-accelerated death, and/or may involve energy-dependent facilitated transport. Which of these factors, if any, controls initially released SON cannot be determined since excretion is a complex phenomenon, and not well understood even in pure cultures. Extrapolation to heterogeneous activated-sludge cultures is useful but difficult. The major observation is that presence of a utilizable carbon source, in some cases at least, stimulates initial release of SON in excess of SON_{eq}.

TABLE 70. SUMMARY OF $\underline{SON_q}$ DATA FOR ALL EXPERIMENTS[a]

Day or Date[b]	Culture: MLSS (mg/l)	Feed Condition	SON_q (mg/l)	SON Technique
8/75-9/75	Palo Alto (PA)	[c]Initial release studies (SON_{eq} = 0.22 ± 0.10 mg/l, n = 8)	0.20 ± 0.09	Technicon & Kjeldahl
9-23-75	PA: 178	200 mg/l glucose COD	0.38	Kjeldahl
220-302	AS Culture 1 (C1)	[c]Initial release studies (SON_{eq} = 0.12 ± 0.04 mg/l, n = 23)	0.11 ± 0.04	Kjeldahl
221	C1: 2040	1000 mg/l glucose-acetate	0.42	Kjeldahl
		Control	0.14	"
98	C1: 362	869 mg/l glucose-acetate COD	0.41	Technicon
"	C1: 370	854 mg/l glucose-acetate COD	0.84	"
"	C1: 352	869 mg/l glucose-acetate COD	0.52	"
"	C1: 350	Control	0.14	"
61	C1: 218	954 mg/l glucose-acetate COD	0.30	Technicon
"	C1: 212	477 mg/l glucose-acetate COD	0.48	"
"	C1: 222	98 mg/l glucose-acetate COD	0.41	"
"	C1: 212	Control	0.09	"
124-173	AS Culture 2 (C2)	[c]Initial release studies (SON_{eq} = 0.06 ± 0.01 mg/l, n = 6)	0.05 ± 0.01	Kjeldahl
171	C2: 615	929 mg/l glucose-acetate COD	0.10	Kjeldahl
"	C2: 575	468 mg/l glucose-acetate COD	0.07	"
"	C2: 565	227 mg/l glucose-acetate COD	0.07	"
"	C2: 565	78 mg/l glucose-acetate COD	0.07	"
168	C2: 2040	670 mg/l glucose-acetate COD	0.04	Kjeldahl
"	C2: 60	670 mg/l glucose-acetate COD	0.05	"
165	C2: 1080	270 mg/l glucose-acetate COD	0.06	Kjeldahl
"	C2: 585	270 mg/l glucose-acetate COD	0.08	"
"	C2: 150	270 mg/l glucose-acetate COD	0.04	"
162	C2: 1450	270 mg/l glucose-acetate COD	0.09	"
"	C2: 760	270 mg/l glucose-acetate COD	0.03	"
156	C2: 2000	270 mg/l glucose-acetate COD	0.11	"
153	C2: 590	300 mg/l glucose-acetate COD	0.16	Kjeldahl

[a] $SON_q = SON_{om} - SON_{oc}$ (Eq. 8).

[b] Day of Operation reported for AS Cultures; date reported for Palo Alto Culture.

[c] Fed tap water; SON_{eq} reported (see Initial SON Release); n = number of samples.

$dSON_t$ Peaks--

Review of Figures 14, 25, 26, 28, and 30 indicates a peak in produced SON occurred within zero to two hours after mixing of feed solutions and activated sludge. The peaks generally occurred prior to the point of complete substrate removal. To test for statistical significance of observed SON peaks, the following equations were used:

$$dSON_{pk} = SON_{peak} - SON_{oc} \tag{22}$$

$$dSON_{pkm} = SON_{peak} - SON_{min} \tag{23}$$

where SON_{peak} is the maximum SON concentration observed during the experiment, SON_{oc} is the initially calculated SON, and SON_{min} is the minimum SON measured after the peak SON had occurred. Differences required for a 95 percent confidence level are listed in Table 71 along with the summary of SON peak data.

TABLE 71. SUMMARY OF DATA DESCRIBING SON PEAKS DURING AERATION

Culture	$SCOD_{oc}$ (mg/l)	$MLSS_{om}$ (mg/l)	$dSON_{pk}$ (mg/l)	$dSON_{pkm}$ (mg/l)	Time from Hour 0 to Peak (hrs.)	Time from Peak to Minimum (hrs.)	SON Technique
AS Culture 1	1000	2040	0.42	0.22	0	0.5	Kjeldahl
AS Culture 1	954	218	0.62	0.62	1	35	Technicon
"	477	212	0.67	0.58	1	7	"
"	98	222	0.61	0.54	1	3	"
"	(Tap) 3	212	0.69	0.67	1	3	"
AS Culture 1	869	362	0.54	0.47	2	10	Technicon
"	854	370	0.84	0.84	0	2	"
"	869	352	0.52	0.46	0	8	"
"	(Tap) 2	350	0.47	0.33	1	7	"
AS Culture 2	929	615	0.15	0.12	1	2	Kjeldahl
"	468	575	0.07	0.03	0	1	"
"	227	565	0.05	0.01	1	2	"
"	976	565	0.11	0.08	0	6	"
AS Culture 2	270	2000	0.15	0.07	3	3	Kjeldahl
"	270	1450	0.09	0.05	0	1	"
"	270	150	0.10	0.08	1	2	"
Palo Alto AS	186	178	0.99	0.62	4	8	Technicon
"	(Tap) 4	178	0.41	0.19	4	20	"

$dSON_{pk}$ required for 95 percent significance level = 0.10 mg/l (Kjeldahl) and 0.28 mg/l (Technicon).

$dSON_{pkm}$ required for 95 percent significance level = 0.08 mg/l (Kjeldahl) and 0.23 mg/l (Technicon).

Of the 18 samples listed in Table 71, 15 values for $dSON_{pk}$ and 14 values for $dSON_{pkm}$ were high enough to be statistically significant, implying that SON was in fact produced in excess of SON_{oc} during initial stages of aeration and then removed to varying degrees as aeration continued. The magnitude of the peak height for the different samples varied, as did peak width. For some systems, the initially measured SON was the maximum observed. The mean $dSON_{pk}$ for AS Culture 1 (0.60 ± 0.13 mg/l) was greater than SON_{eq} (0.12 ± 0.04 mg/l) at a 99 percent confidence level. A similar difference could not be demonstrated statistically for AS Culture 2 which had measured SON values near the analytical limit.

The production of SON peaks during substrate oxidation might be the result of a particular phase of growth or rate of growth. However, tap water control systems exhibited similar peaking behavior, and no statistically significant difference between levels of produced SON for these systems and those fed exogenous carbon was found. Thus, presence of external carbon sources and resulting differences in growth rate or phase of growth cannot alone explain the produced SON; other factors must also be involved.

The only significant difference found between the behavior of systems fed a utilizable carbon source and those fed tap water was that initially released SON from carbon-fed systems with some cultures was greater. This increase is directly attributable to SON_g. The major observation is that SON is released rapidly by cells immediately after dilution with tap water or a new substrate, a peak concentration of SON is reached within a few hours, after which the concentration decreases. This behavior is consistent and is affected by factors other than growth rate.

DISCUSSION OF RESULTS

Sources of Effluent SON

The SON contained in activated-sludge effluents (SON_e) has two general sources: (1) influent SON (SON_i) that is not removed during treatment, and (2) SON produced during treatment (SON_p). SON_i contains both biodegradable (SON_b) and refractory material (SON_r), as does produced SON (SON_{pb} and SON_{pr}, respectively). SON_p includes an equilibrium concentration (SON_{eq}), SON produced by organism decay (SON_d), and SON produced as a result of substrate oxidation, i.e., due to organism growth (SON_g). SON_p is defined by a modified form of Eq. 3:

$$SON_p = SON_{eq} + SON_d + SON_g \tag{24}$$

Removal of SON_i, production of SON_p, and the contribution of these processes to SON_e are discussed below.

SON Production

Conceptual Model of SON_p--

A conceptual model of changes in SON_p during batch activated-sludge treatment can be developed from experimental data (Figures 14, 16, 17, 18, 25, 26, 28, and 30), and supported by fundamental theory (see "SON Production and Excretion by Bacteria"). This SON production function is meant to provide a framework for discussing changes in produced SON sources during activated-sludge treatment, and is not meant to be quantitative or absolute. It is a composite of observed experimental behavior. Reference to the model, along with presentation of relevant supporting data and fundamental theory, will be made when discussing SON_p, SON_{eq}, SON_d, and SON_g.

SON_p--

Although growth rate (μ) could not be mathematically correlated to SON production during start-up of semi-continuous fed laboratory Activated Sludge (AS) Cultures (Figures 16, 17, and 18), μ probably exerted an effect on SON_p levels. The peak SON concentration (up to 5 mg/l) was observed when MLSS was less than 1500 mg/l. At these lower MLSS levels, longer aeration periods were required to remove added glucose-acetate substrate during the 23-hour daily feed cycle. Growth rate thus remained high for a longer period and resulted in continued production and excretion of SON metabolites. As MLSS levels increased to near 1500 mg/l, starvation conditions were reached earlier in the feed cycle and degradable SON_p excreted during substrate unlimited conditions was utilized as expected [101,148,199]. Experiments utilizing chemostats with varying θ_c (1/μ) over a wide range (0.5 to 20 days) would be needed to reliably determine the effect of μ on SON_p. However, the semi-continuous data do indicate that operation under unlimited substrate conditions may result in high concentrations of produced SON.

Significant differences in SON_p concentration were associated with changes in AS Culture characteristics. These differences occurred during steady-state operation ($\mu = 0.17$ day^{-1}). This is an important observation because it implies that even if growth rate can be controlled to minimize SON_p for one set of conditions, significant changes in SON_p may still occur as a result of natural culture population shifts. The optimal growth rate may also shift.

Fluctuation in SON_p levels during activated-sludge treatment may primarily be due to differences in production of biodegradable SON. Estimates of refractory SON were similar for AS Culture 2 during start-up, AS Culture 2 at steady-state, and AS Culture 1 at steady-state (0.40, 0.26, and 0.14 mg/l, respectively). These differences were statistically significant but small when compared to differences in SON_{pb}. Values of SON_{pb} for the three samples were 1.63, 0.62, and 0 mg/l, respectively. AS Culture start-up corresponds to operation at aeration times less than t_{min} (Figure 32), while steady-state operation corresponds to aeration times greater than t_{min}. Operation to the left of t_{min} will likely result in production of higher concentrations of degradable SON than aeration times greater than t_{min}. Variability in SON_{pb} levels for systems operating to the right of t_{min} (steady-state samples) again illustrates perturbations in SON production under identical operating conditions.

Peak SON_p concentrations (Figure 32) observed during batch studies were real, but variable for different activated-sludge cultures (0.10 to 0.60 mg/l). Variability in peak SON_p was probably due to differences in cultural characteristics. Peak heights were independent of SCOD, MLSS, and NH_3-N

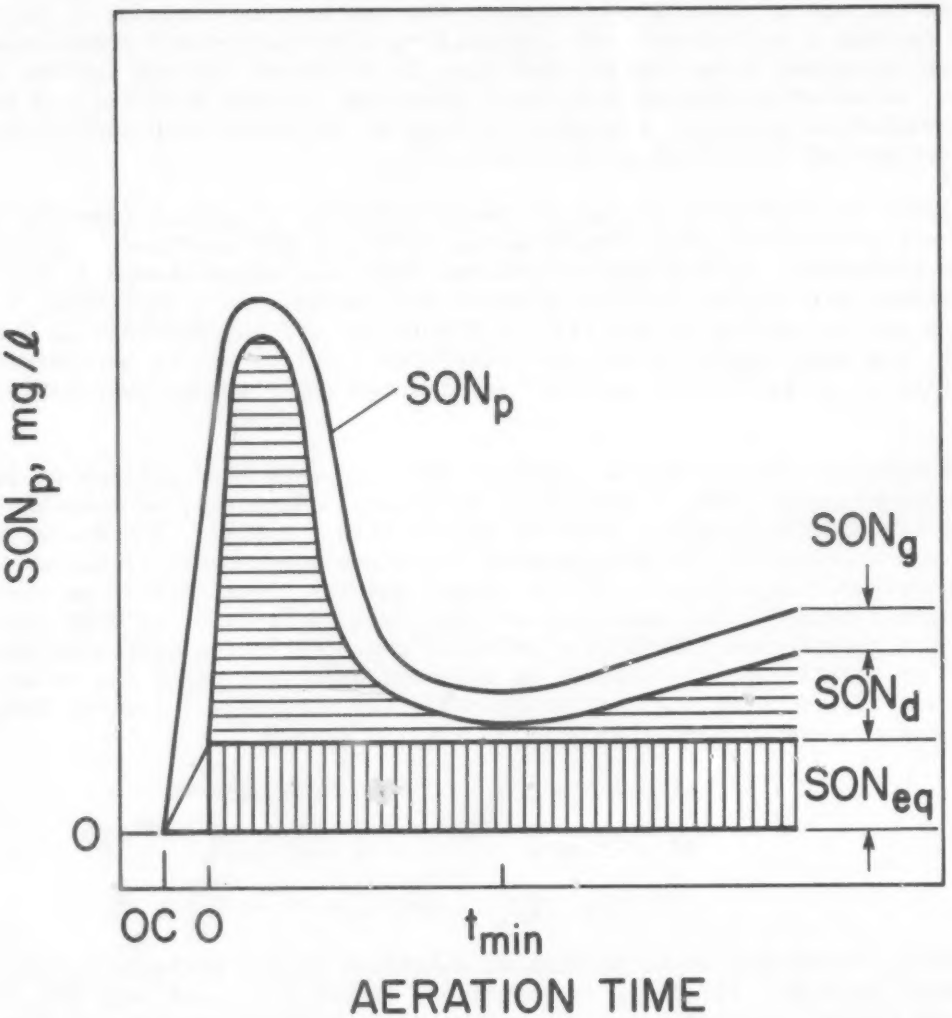

SON$_p$, mg/ℓ
SON$_p$
SON$_g$
SON$_d$
SON$_{eq}$
O
OC
O
t$_{min}$
AERATION TIME

Figure 32. Conceptual model of changes in SON$_p$, SON$_g$, SON$_d$, and SON$_{eq}$ with batch activated-sludge aeration time.

concentrations, and substrate type. The SON produced in peak concentration was degradable since it was removed to a significant degree as aeration continued. Peaking behavior is explained by bacterial production and utilization. Bacteria produce and excrete SON in response to concentration gradients, starvation conditions, addition of exogenous substrate, and changes in phase and rate of growth. Produced SON is utilized by other organisms in the heterogeneous culture as aeration continues. The utilization response is fairly rapid (within 2 to 4 hours) and a general equilibrium between production and utilization seems to be reached near t_{min} (~ 4-8 hours for the systems studied). As aeration time exceeds t_{min}, substrate becomes limiting and starvation conditions prevail. A gradual release of SON under such conditions is well documented [99,100,109,153,157,177,178].

Based on discussion presented above, hydraulic detention time (θ) is an important process variable affecting the levels of SON produced by activated-sludge treatment. Variations in influent flow (Q), which change θ, may be responsible for fluctuations in produced SON levels. As Q increases, θ decreases and is shifted to the left in Figure 32. If the decrease is large enough, the peak region of the SON production function may be encountered, resulting in a significant increase in produced SON (largely degradable).

SON_{eq}--

Activated-sludge organisms excrete SON compounds to establish an equilibrium concentration (SON_{eq}) with these particular compounds, an observation consistent with information given by others [150,151,152]. The discovery of SON_{eq} was a result of the experimental procedure used. Concentrated activated-sludge culture was diluted with tap water, and SON_{eq} was defined as the SON initially released. The magnitude of SON_{eq} was independent of MLSS (10-2400 mg/l) and temperature (10-30°C). Cultural characteristics were once again linked to significant differences in SON concentrations since the value of SON_{eq} was dependent on cultural characteristics. Average values of SON_{eq} for the three sludges studied are:

AS Culture 1	0.12 ± 0.04 mg/l
AS Culture 2	0.06 ± 0.01 mg/l
Palo Alto AS	0.22 ± 0.10 mg/l

SON_{eq} is assumed to be present at all times during activated-sludge treatment because: (1) SON_{eq} is refractory (Section 9), and (2) SON_p levels measured during organism decay and synthetic feed batch experiments did not decrease below the SON_{eq} level for any aeration time. There is some question as to the appropriateness of including SON_{eq} as part of SON_p, since during steady-state operation of continuously fed activated-sludge systems, SON may not be "produced" as such. However, it will always be present. As conditions in the aeration basin change, for example, when population shifts occur, SON_{eq} will be adjusted, increasing or decreasing depending on the situation. The major point is that SON_{eq} is part of SON_e. For purposes of this discussion, SON_{eq} is considered to be part of the fraction of SON_e represented by SON_p.

SON_{eq} may be considered refractory with respect to removal by activated sludge, but may be degradable under some circumstances. Since its magnitude is dependent on cultural characteristics, SON compounds contained in SON_{eq} for one organism may not be included in SON_{eq} for other organisms. A portion of SON_{eq} from one organism may be removed when population shifts occur and other organisms become predominant. If for some reason organisms were exposed to higher concentrations of the nitrogenous components comprising SON_{eq}, the additional SON should be removed until SON_{eq} is reached. A limited experiment showed that exposure of AS Culture to concentrated SON_{eq} ($\sim 2\ SON_{eq}$) resulted in some SON removal. However, additional experiments would be needed to reach a firm conclusion. For purposes of discussing SON_e, SON_{eq} will be considered refractory, since Biodegradation Study 3 (Section 9) showed it to be 100 percent refractory after 30 days degradation.

SON_d--

SON produced due to organism decay (SON_d) was defined to be the SON released in excess of SON_{eq} during batch aeration of activated-sludge cultures with tap water. SON_d was calculated from Eq. 25:

$$SON_d = dSON_t - SON_{eq} \qquad (25)$$

in which $dSON_t$ is the SON released at a given aeration period (t). Relationships for $dSON_{23}$ were determined directly for AS Cultures 1 and 2, and can be estimated for Palo Alto culture using the equations listed in Table 68. The method for deriving the Palo Alto culture relationship is described later. The resulting relationships are:

AS Culture 1 $dSON_{23} = 0.05 + 0.00008\ MLSS$

AS Culture 2 $dSON_{23} = 0.02 + 0.00008\ MLSS$

PA Culture $dSON_{23} = 0.22 + 0.00020\ MLSS$

It is apparent that the contribution of SON_d to SON_p varies with culture characteristics, an observation similar to that noted for SON_{eq}.

The shape of the SON behavior curve for most organism decay studies approximated the conceptual SON_d curve shown in Figure 32. Release of SON was a response to starvation conditions in which degradation of intracellular RNA and protein occurs with accompanying excretion of some of the degradation products. Peaking of SON_d may have occurred because the excretion rate of degradation products was greater than utilization rate for a short period after the organisms were exposed to the new environment (tap water). After a period of acclimation (2 to 4 hours), SON_d decreased due to utilization of excreted, biodegradable SON by remaining viable cells [101,199]. The gradual increase in SON_d after t_{min} was probably due to excretion of cellular degradation products that were not utilized at the same rate they were produced. This material was refractory. The gradual increase was expected based on reports by others [99,100,109,153,157,177,178], and was approximately linear with aeration time. A very slow increase in SON was also noted for laboratory aerobic digesters developed with AS Culture.

SON_d for aeration times greater than t_{min} was strongly correlated with MLSS concentration; increased SON concentrations were associated with higher MLSS concentrations. An important implication of this observation is that operation of activated-sludge systems at high MLSS values and a given waste detention time should result in production of larger quantities of refractory SON than will lower MLSS systems. The dependence of SON_d on MLSS can be explained by differences in SON production and utilization rates as discussed above. Assuming the net SON_d production rate (production - utilization) to be constant for a culture of organisms, larger concentrations of organisms will result in more net production of SON_d.

Whether the SON_d peaks described by Figure 32 would be observed with continuously fed activated-sludge systems is open to speculation. During normal activated-sludge operation where solids recycle is practiced, organisms are starved while in the secondary sedimentation basin and the recycle line. When these starved organisms are suddenly diluted upon re-entry to the aeration basin, a portion of the organisms will remain starved, and excrete SON as described above. Other viable organisms may rapidly remove the excreted SON such that a peak may not be observed. Appropriate chemostat experiments would have to be conducted to adequately determine if SON_d peaks would appear at lower values of θ. Reliable estimates for the contribution of SON_d to SON_p are difficult because production and excretion of SON by heterogeneous activated sludge is complex. The approach used during this research, although not without limitation, did point out that the contribution of SON_d may be quite significant at short aeration times.

SON_g--

SON_g, estimated from Eq. 26, was not significantly greater than zero for any batch experiment except Batch Study 6:

$$SON_g = dSON\ (substrate) - SON_d - SON_g \qquad (26)$$

dSON (substrate represents the SON produced by an activated-sludge system fed a utilizable substrate. The only consistently significant difference between SON_g and $SON_d + SON_g$ was measured at time zero for AS Culture 1. SON_p values for AS Culture 2 were so near the analytical limit that significant differences could not be detected. Two interpretations are possible from these observations: (1) SON_g is in fact zero for aeration times greater than time zero, and (2) estimation of SON_d is erroneously high and SON_g is not really zero.

If SON_g is zero, it means growth rate had no effect on SON production and most of the SON produced during the batch experiment was the result of organism decay. Data from the literature suggest that this should not be the case; substrate unlimiting conditions should result in excretion of larger concentrations of SON than starvation conditions [101,113,148]. The fact that initially released SON for substrate-fed systems was significantly higher than for tap control systems (for AS Culture 1) implies that substrate has an effect. Perhaps organisms utilized excreted SON at a faster rate in the substrate-fed systems thus depressing the SON_p peak height to a level experimentally indistinguishable from the SON_d peak.

It could not be determined from experimental data whether the method used to estimated SON_d resulted in erroneously high values. A satisfactory alternative method for estimating SON_d could not be found. The major observation was that SON was produced rapidly by organisms when added to tap water or tap water plus substrate, and then removed to a significant degree. In general, no significant difference between SON levels produced by these two systems was discernible, that is, SON_g may be zero as measured experimentally.

Contribution of SON_{eq}, SON_d, and SON_g to SON_p--
The contribution of the individual sources of SON to SON_p was estimated. Methods used in making this estimation are presented below.

1. $\underline{SON_p}$--Steady-state, AS Culture effluent SON concentrations (Table 59) were used to estimate SON_p. Two different estimates with different assumptions were made. In the first estimate, feed solution SON (SON_f: 0.10 ± 0.04 mg/l) was assumed to be completely refractory and SON_p was calculated as follows:

$$SON_p = SON_e - SON_f$$

where SON_e represents the steady-state effluent SON at the end of the 23-hour daily feed cycle. For the second estimate, SON_f was assumed to be completely degradable, making SON_p equal to SON_e. The true value for SON_p is probably somewhere between these two estimates. Table 72 lists the values of SON_p which resulted.

2. $\underline{SON_{eq}}$--Values of SON_{eq} used were discussed earlier and are listed in Table 72.

3. $\underline{SON_d}$--Since 23-hour measurements for SON_e were used, the relationships developed previously for 23-hour estimates of SON_d are appropriate. These relationships are:

AS Culture 1 $\quad SON_d = 0.05 + 0.00008\ MLSS - SON_{eq}$, e = 0.02

AS Culture 2 $\quad SON_d = 0.02 + 0.00008\ MLSS - SON_{eq}$, e = 0.02

where e is the standard error of estimate. The MLSS values listed in Table 59 were used to estimate SON_d. These estimates are contained in Table 72.

4. $\underline{SON_g}$--Estimates for SON_g (Table 72) were calculated using the rearranged form of Eq. 24 listed below:

$$SON_g = SON_p - SON_{eq} - SON_d$$

Use of this method for estimating SON_g was both necessary and reasonable, since experimentally determined values of SON_g were not significantly different from zero.

Although the proposed method is not eithout limitations, it should yield

TABLE 72. CONTRIBUTION OF $\underline{\text{SON}}_{eq}$, $\underline{\text{SON}}_{d}$, AND $\underline{\text{SON}}_{g}$ TO $\underline{\text{SON}}_{p}$ FOR AS CULTURES

Sample (see Table 5.3)	SON_p (mg/l)[a]	SON_{eq}		SON_d		SON_g	
		mg/l[a]	Percent[b] of SON_p	mg/l[a]	Percent[b] of SON_p	mg/l[a]	Percent[b] of SON_p
Assume SON_f is refractory:							
AS Culture 1 - Data Set 1	0.23 ±0.06	0.12 ±0.04	52 ±22	0.09 ±0.04	39 ±20	0.02 ±0.07	9 ±32
AS Culture 1 - Data Set 2	0.53 ±0.19	0.12 ±0.04	23 ±11	0.11 ±0.04	21 ±11	0.30 ±0.19	57 ±41
AS Culture 2	0.16 ±0.06	0.06 ±0.01	38 ±16	0.13 ±0.02	81 ±33	-0.03 ±0.07	-
Assume SON_f is degradable:							
AS Culture 1 - Data Set 1	0.33 ±0.06	0.12 ±0.04	36 ±14	0.09 ±0.04	27 ±13	0.12 ±0.07	36 ±22
AS Culture 1 - Data Set 2	0.63 ±0.19	0.12 ±0.04	19 ±9	0.11 ±0.04	17 ±8	0.40 ±0.19	63 ±31
AS Culture 2	0.26 ±0.06	0.06 ±0.01	23 ±7	0.13 ±0.04	50 ±19	0.07 ±0.07	27 ±28

[a] ± values are standard deviations.

[b] ± values are standard deviations for percent as calculated by method similar to that described in Appendix B, item 8-e.

reasonable ranges for percent of SON_p contained in the SON_{eq}, SON_d, and SON_g fractions. Results are summarized in Table 72.

Caution must be used in interpreting the results listed in Table 72 because SON concentrations were, for the most part, low and near the analytical limit. However, some general comments can be made. SON_{eq} constitutes a significant fraction of produced SON, and since it is expected to be present at all times during treatment, cannot be effectively controlled or minimized. The magnitude of SON_d was small, and nearly equal for the three samples. SON_g was essentially zero for two data sets, but constituted the major fraction of SON_p for the other data set. SON_p was higher for this data set, exhibited more fluctuations (Table 59, Figure 18), and most likely contained a high proportion of biodegradable SON (Biodegradation Sutdy 5). These data for SON_g and SON_d agree with observations discussed earlier suggesting that fluctuations in SON_p levels may be due to fluctuations in degradable SON production. These fluctuations are in turn linked to cultural characteristic differences.

Quantitative estimation of the relative contribution of the individual SON sources to SON_p at aeration times other than 23 hours can be made by referring to Figure 32. As aeration time is decreased below t_{min}, the relative contribution of SON_d is expected to decrease. However, as discussed earlier, the uncertainty in the estimate of SON_d at lower aeration times makes definitive statements difficult. Perhaps a better way to describe relative contributions would be to say that the contribution of $SON_d + SON_g$ increases as aeration times below t_{min} should result in increased production of SON.

Changes in SON_e during Activated-Sludge Treatment of Domestic Wastewater

Evaluation of influent SON (SON_i) removal from untreated domestic wastewaters is complex because production of SON and removal of this produced SON occurs concurrently with removal of SON_i. Separation of these processes is difficult. However, SON_e is readily measured. In the following, factors affecting changes in SON_e (removal of produced SON plus SON_i) are discussed, a conceptual model illustrating the potential SON_e sources is presented, individual SON sources are briefly discussed, and finally the contribution of each SON source to SON_e is estimated. The Palo Alto activated-sludge system is used as the example for these estimates. Data from the AS culture experiments are used to aid in the evaluation.

Factors Affecting Changes in SON_e--

Factors affecting the decrease in SON from primary effluent in laboratory batch studies using Palo Alto activated sludge were aeration time and MLSS concentration. There was an aeration time (t_{min}) at which SON_e reached a minimum concentration and beyond which it increased, primarily due to SON production from organism decay. This increase in SON beyond t_{min} up to about 48 hours was approximately linear with time, and was also a linear function of MLSS concentration. A similar observation was noted for SCOD, a result also reported by others [62,212,213,214,215,216]. The value of t_{min} was a function of MLSS, ranging from 6 hours for a 1200-mg/l MLSS to 48 hours for a 180-mg/l system. The organics present at minimum SON and SCOD values were 100 percent refractory for one batch study. The decreases in SON concentration below the influent value at t_{min} were 62 ± 6 percent, giving an effluent

SON (SON_e) of 1.40 ± 0.46 mg/l for the eight samples tested, results similar to those found for the full-scale Palo Alto plant (Section 6). SON and SCOD removal rates increased as MLSS was increased.

SCOD concentration in general decreased more rapidly than SON concentration. Differences in the nature and source of these materials can in part explain removal rate differences. Characterization studies showed that (1) SCOD decreased at a faster rate (relative rates were about 0.26 day^{-1} for SCOD and 0.13 day^{-1} for SON), (2) apparent molecular weight distributions for SON and SCOD were different, and (3) ion-exchange behavior of the SON and SCOD were different. In addition, relative production of SON during batch aeration may be greater than relative production of SCOD, resulting in depression of the net SON removal rate relative to that of SCOD. This effect was most dramatic for the 1.4-and 14-mg/l MLSS systems for Batch Study 3 in which SON actually increased during initial stages of aeration while SCOD decreased.

Conceptual Model of SON Removal--

It is helpful to construct a conceptual model of net SON removal during activated-sludge treatment to better understand SON behavior. The model should include the production function described under SON production, along with a description of changes in influent degradable (SON_b) and refractory (SON_r) fractions during treatment. Such a model is shown in Figure 33, and serves as a basis for discussing the sources of effluent SON. The shape of the SON_e curve is a composite of experimental behavior observed during batch removal studies with Palo Alto activated sludge.

The model is not without limitations. Experiments were with batch systems in which primary effluent was mixed with Palo Alto activated sludge. Measured time zero SON and SCOD concentrations for systems with MLSS levels near 1000 mg/l were 10 to 20 percent lower than in solutions without MLSS as uptake occurred during processing (15 minutes) of the time zero sample. Release of SON_g and release of SON to reach SON_{eq} (see Figure 32) also took place during this 15-minute period. After time zero, SON behavior was measurable and consistent. Figure 33 and subsequent discussion of SON_e are based on observed behavior for time periods after initial mixing.

As drawn, Figure 33 represents results from a relatively high MLSS system (> 1000 mg/l). For lower MLSS values, the decrease in SON_e would be slower (net removal rate less), and if MLSS were low enough, SON_e may actually increase during initial stages of aeration due to SON production (see 1.4- and 14-mg/l MLSS systems, Batch Study 3). This aspect will be discussed in detail under $SON_d + SON_g$.

Individual Sources of SON_e--

Sources contributing to SON_e include SON_i ($SON_b + SON_r$), SON_{eq}, SON_d, and SON_g.

1. $\underline{SON_i}$--SON_i was shown to be highly degradable; more than 80 percent of SON_i may be represented by SON_b. SON_b will comprise the major fraction of SON_e during early stages of aeration, but may be removed almost completely as aeration continues (see Biodegradation Study 3). Thus, it is not likely that SON_b will comprise a significant fraction of SON_e if sufficient detention

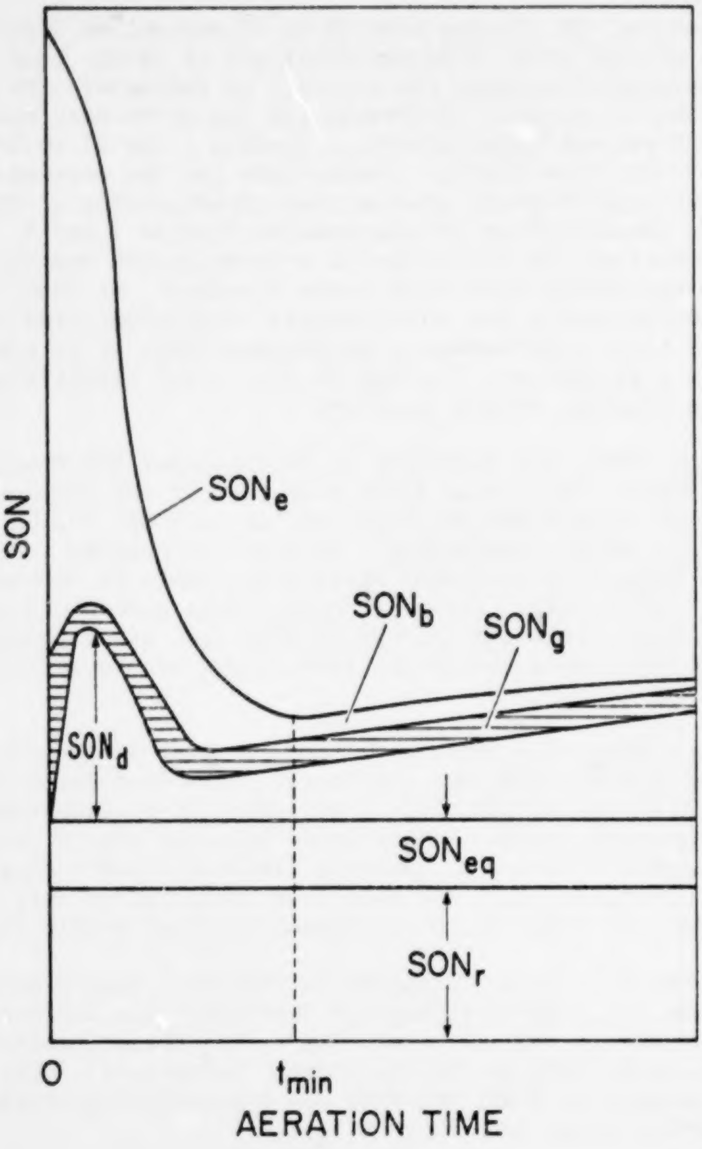

SON
SONe
SONb
SONg
SONd
SONeq
SONr
0
tmin
AERATION TIME

Figure 33. Conceptual model of changes in sources of effluent SON as a function of activated-sludge aeration time.

time is provided. The rate at which SON_b is removed during treatment increases as MLSS concentrations increase.

Estimates for SON_r ranged from 18 to 38 percent of SON_i. Reliable approximations of the "true" SON_r are difficult to obtain from low seed biodegradation experiments because the quantity of refractory SON produced during the time period is unknown. Estimates for the refractory portion of SON_e ranged from 40 percent (Biodegradation Studies 1 and 2) to 100 percent (Biodegradation Study 3 and [202]). Predictions for the concentration of SON_r were sometimes significantly greater than corresponding predictions for refractory SON_e concentration (Biodegradation Studies 2 and 3). This apparent dichotomy emphasizes the complexity in separating SON sources. Perhaps some SON_r was removed during activated-sludge treatment, or more likely, additional SON_r was produced during the biodegradation experiment used to predict SON_r. Regardless of these limitations in estimating SON_r, it is apparent that SON_r will comprise a significant fraction of SON_e. For simplification, SON_r is assumed to be constant during aeration.

2. $\underline{SON_{eq}}$--SON_{eq} was described in detail under SON Production. Primary wastewater already contains at least a portion of the compounds comprising SON_{eq}. As such, they would be "measured" as part of SON_r because SON_{eq} was found to be 100 percent refractory. However, if present in concentrations greater than SON_{eq}, the increment above SON_{eq} would be removed and hence measured as part of SON_b. Since it could not be determined what fraction of SON_{eq}, if any, was contained in SON_r or SON_b, and since reasonably reliable estimates for SON_{eq} were easily obtained, SON_{eq} was considered a separate source of SON_e.

3. $\underline{SON_d + SON_g}$--For reasons alluded to under SON Production, reliable separation of SON_d and SON_g was difficult. Data from Batch Study 4 with Palo Alto activated sludge support this observation by showing that the only significant differences between SON produced by a tap control system and a glucose-fed system occurred at aeration times of 2 and 4 hours (corresponding to peak SON concentrations), and even then, differences were relatively small. Therefore, SON_d and SON_g will be discussed together rather than individually.

$SON_d + SON_g$ include a biodegradable fraction, most prevalent at aeration times less than t_{min}, and a refractory fraction. The refractory fraction ranges from 20-100 percent of total $SON_d + SON_g$ (Biodegradation Studies 4 and 5 with AS culture); SON_d may be 100 percent refractory. $SON_d + SON_g$ exhibited a linear dependence on MLSS, and SON_d was approximately linear with aeration time for aeration times greater than t_{min}.

Direct evidence for the contribution of $SON_d + SON_g$ to SON_e comes from Batch Study 3 data (MLSS = 1.4, 14 mg/l). SON_e increased by up to 0.6 mg/l while SCOD was being removed (10-20 percent) during the first six hours of aeration of the primary effluent with Palo Alto activated sludge. Acclimation or lag phenomena do not explain the increase in SON since SCOD was being removed. The magnitude of the increase (0.4 to 0.6 mg/l) was similar to the average peak SON produced by AS Culture 1 systems.

At some time during the first 4 hours of treatment, SON_e increased, or at least remained constant, for all batch systems studied. If no SON were produced during treatment, the SON removal curve should be a relatively smooth, continuously decreasing curve similar to that predicted by classical Monod kinetics for organic removal [200], but it was not.

The contribution of $SON_d + SON_g$ to SON_e varies with changes in culture characteristics. For example, peak production of $SON_d + SON_g$ was 0.48 ± 0.14 mg/l for AS Culture 1 but only 0.04 ± 0.04 mg/l for Culture 2 (both fed identical glucose-acetate substrates). During Batch Study 4, with Palo Alto AS, peak $SON_d + SON_g$ was 0.77 mg/l for the system fed glucose. A major finding of this study is that variability in SON behavior which is related to "uncontrollable" culture characteristics.

Estimation of Contribution of SON Sources to Palo Alto Activated-Sludge SON_e--

It is difficult to quantitatively model net SON removal because of the complex nature of the SON production function. The data required for deriving a fundamental mathematical description of the production function could not be obtained because produced SON values were so low for the AS Cultures developed, especially for AS Culture 2. Therefore, a general qualitative description of the phenomena is given with estimated ranges of the percent contributions of the potential SON sources to Palo Alto SON_e. These ranges are widely variable due to fluctuations in experimental data, analytical limitations due to low SON concentrations, and difficulty in experimentally separating the individual SON sources. However, the data do support several important findings of this study: (1) there are several sources of SON significantly contributing to SON_e, (2) these sources contribute to SON_e in variable quantities depending on cultural characteristics, and (3) SON behavior during activated-sludge treatment is complex.

One of the most significant aspects of this study was the discovery of SON_{eq} as a possible source of SON_e. Its general contribution to SON_e can be estimated by comparing SON_{eq} values obtained from laboratory experiments using Palo Alto activated sludge (0.22 ± 0.10 mg/l for 8 samples tested in 7/75-10/75) with the average Palo Alto SON_e (1.33 ± 0.28 mg/l for 22 samples analyzed from 1/75-12/75)(Section 6). Thus SON_{eq} is 17 ± 8 percent of SON_e. SON_{eq} will vary with cultural characteristics and may sometimes be very small, as was the case for AS Culture 2 ($SON_{eq} = 0.06 \pm 0.01$ mg/l; 5 percent of the average Palo Alto SON_e). SON_{eq} is significant in that it is always a portion of SON_e and is not subject to direct activated-sludge operational control.

Three estimates for the contribution of individual SON sources to Palo Alto SON_e were obtained using data from batch studies with Palo Alto activated sludge. SON_{eq} was assumed to be 0.22 ± 0.10 mg/l in all estimations.

In the first estimate a high MLSS system was used (Batch Study 3, 1390 mg/l MLSS) with contributions to SON_e at aeration times equal to or greater than t_{min}. $SON_d + SON_g$ was estimated using the equations developed from tap controls listed in Table 68. Substituting the average SON_{eq} in these equations, and noting that the MLSS coefficient was linear with aeration time, the following general equation was derived for produced SON at any aeration time t ($dSON_t$):

$$dSON_t = 0.22 + [0.0001 + (4.17)(10^{-6})(t)]\ MLSS \qquad (28)$$

The estimated concentration of SON_d + SON_g then becomes $dSON_t$ minus SON_{eq}. The standard error of estimate for this equation, 0.15 mg/l, was high due to possible analytical error in the 24-hour SON measurement (Table 68). SON_r was estimated as 20 percent of the initially calculated SON for the primary-fed system. SON_b was estimated by subtracting SON_{eq}, SON_d + SON_g, and SON_r from measured SON_e. Results for this estimate are summarized in Table 73.

The release of SON due to organism decay becomes more significant (up to 21 percent) as aeration time was increased, an expected occurrence. SON and SCOD produced were expected to be refractory (see Biodegradation Study 3). A limitation of this estimate is the inability to accurately determine SON_r and SON_b. By definition, SON_b cannot increase since it was the biodegradable portion of the influent. Limitations in the estimate of SON_r have already been discussed. Perhaps the increase in SON_b after t_{min} was in fact due to increases in SON_d + SON_g from substrate oxidation that were not observed in the tap water control system used to estimate SON_d + SON_g. The data show that the contribution of SON_d + SON_g was 13 to 21 percent for the listed aeration times.

The second estimate was obtained from Batch Study 4 data; MLSS was about 180 mg/l. This experiment represented an attempt to estimate the contribution of SON_d + SON_g to Palo Alto SON_e by feeding a glucose substrate to Palo Alto culture and comparing this system with one fed primary effluent. SON_d + SON_g was calculated using the glucose-fed system and correcting for the contribution of SON_{eq} and SON_{oc} (initially calculated SON). SON_{eq}, SON_r, and

TABLE 73. ESTIMATION OF $\underline{SON_{eq}}$, $\underline{SON_d}$ + $\underline{SON_g}$, $\underline{SON_r}$, AND $\underline{SON_b}$ IN PALO ALTO SECONDARY EFFLUENT
(MLSS = 1390 mg/l)

Aeration Time (hours)	Measured SON_e* (mg/l)	SON_b (percent of SON_e)*	SON_d + SON_g (percent of SON_e)[1]	SON_r (percent of SON_e)*	SON_b (percent of SON_e)*
0	3.16 ± 0.16	7 ± 3	4 ± 4	23 ± 5	66 ± 9
6 (t_{min})	1.30 ± 0.16	17 ± 8	13 ± 11	57 ± 14	13 ± 22
12	1.66 ± 0.16	13 ± 8	13 ± 9	45 ± 10	29 ± 17
24	1.81 ± 0.16	12 ± 6	15 ± 8	41 ± 9	32 ± 15
48	1.98 ± 0.16	11 ± 5	21 ± 8	37 ± 8	31 ± 14

* ± values are 95 percent confidence limits.

[1] ± values are estimated derivations based on standard error of estimate (Eq. 26) and 95 percent CI of SON_e.

TABLE 74. ESTIMATION OF $\underline{SON}_{eq}$, $\underline{SON}_d$ + $\underline{SON}_g$, $\underline{SON}_r$, AND $\underline{SON}_b$ IN PALO ALTO SECONDARY EFFLUENT ($\underline{MLSS}$ = 180 mg/1)

Aeration Time (Hrs.)	Measured SON_e (± 0.16)† (mg/1)	SON_{eq} (± 0.08)†		SON_d + SON_g (± 0.29)†		SON_r (± 0.16)†		SON_b (± 0.38)†	
		mg/1	Percent of SON_e*	mg/1	Percent of SON_e*	mg/1	Percent of SON_e*	mg/1	Percent of SON_e*
0	3.93	0.22	6 ± 2	0.16	4 ± 7	0.96	24 ± 4	2.61	66 ± 10
1	3.90		6 ± 2	0.03	1 ± 10		24 ± 4	2.71	69 ± 10
2	3.15		7 ± 3	0.40	13 ± 9		30 ± 5	1.59	50 ± 12
4	2.89		8 ± 3	0.77	27 ± 10		33 ± 6	0.96	33 ± 13
8	2.46		9 ± 3	0.21	9 ± 12		37 ± 7	1.07	44 ± 16
12	1.91		12 ± 4	0.15	8 ± 15		50 ± 9	0.60	31 ± 20
24	1.85		12 ± 4	0.24	13 ± 16		51 ± 10	0.45	24 ± 21
48	1.62		14 ± 5	0.46	28 ± 18		59 ± 11	-0.02	(-1)**
72	1.71	0.22	13 ± 5	0.67	39 ± 17	0.96	56 ± 11	-0.14	(-8)**

† ± values are 95 percent confidence limits (mg/1).

* ± values are 95 percent confidence limits for percent contribution.

** See text for explanation.

SON_b were estimated as in the previous example. Results are summarized in Table 74, along with 95 percent confidence limits for estimated values. Confidence limits for $SON_d + SON_g$ and SON_b were high since subtraction of several SON measurements were used to obtain these estimates.

Despite the relatively high uncertainties for the estimates listed in Table 74, the general shape of the conceptual SON_e behavior model was approximated. The contribution of $SON_d + SON_g$ is maximum near the peak of the production function and at long aeration times. $SON_d + SON_g$ present after long periods of aeration will likely be refractory. The contribution of SON_b, although somewhat erratic, is highest during initial stages of aeration. SON_r constitutes the major fraction after long periods of aeration.

As previously discussed, inability to accurately determine SON_r limits the estimation of SON source contribution. Higher estimates for SON_r would have resulted in SON_b values much less than zero (in this case, for 48- and 72-hour data, SON_b values were slightly negative), while lower estimates would yield high values for SON_b at long aeration times. Changes in the estimate of $SON_d + SON_g$ would also change SON_b estimates. Separation of SON production from SON_i removal is clearly a complex undertaking. This particular attempt, one employing reasonable assumptions, showed that 20 to 50 percent of SON_e may be produced during treatment.

For the third estimate, the contribution of the individual SON sources to Palo Alto SON_e at the aeration time corresponding to minimum SON was estimated by combining data from all Batch Studies using Palo Alto activated sludge. $SON_d + SON_g$ was estimated from tapwater control systems and the glucose-fed system of Batch Study 4, and SON_r, SON_b, and SON_{eq} were determined as described earlier. Estimations are summarized in Table 75.

The estimate of SON_b may again be erroneously high due to the inability to separate SON_g from SON_d. Within the recognized limitations, it is still reasonable to state that (1) SON_{eq} was a significant source of minimum SON_e, (2) SON produced due to organism decay (SON_d) is significant even at minimum SON_e, and (3) SON_r represents the major fraction of minimum SON_e.

TABLE 75. ESTIMATION OF $\underline{SON_{eq}}$, $\underline{SON_d} + \underline{SON_g}$, $\underline{SON_r}$, AND $\underline{SON_b}$ IN PALO ALTO SECONDARY EFFLUENT AT t_{min}

SON_e mg/1	SON_{eq}		$SON_d + SON_g$		SON_r		SON_b	
	mg/1	% of SON_e	mg/1	% of SON_e	mg/1	% of SON_e	mg/1	% of SON_e
1.40±0.46 (n=8)	0.22±0.10 (n=8)	16 ± 9	0.26±0.16 (n=7)	19 ±13	0.73±0.17 (n=8)	52 ±2	0.19±0.53	14 ±39

n = number of samples used for estimation.

± are standard deviations for combined data.

Although it was difficult to experimentally separate and determine precisely the contribution of individual SON sources to Palo Alto activated-sludge effluent SON, reasonable estimates were obtained. Aeration times of about 6 hours and MLSS values of 1200-1500 mg/l are typical for this plant. For these conditions, using batch study data as a basis for estimation, SON_{eq} may account for around 14 to 17 percent of SON_e; SON_d + SON_g may account for 13 to 19 percent of SON_e; SON_r may account for 50 to 60 percent of SON_e; and the remainder is SON_b.

SECTION 9

SON CHARACTERIZATION

INTRODUCTION

The objective of the characterization studies was to determine the nature of the nitrogen-containing soluble organics in selected wastewaters in order to help evaluate the source of SON in activated-sludge effluents. Characteristics evaluated included biodegradability, molecular weight distribution, and free and combined amino acid content.

Wastewaters Characterized

Several treated and untreated municipal wastewater samples from studies described in Section 8 were characterized as listed in Table 76.

Biodegradability

Biodegradation studies were used to estimate the refractory fractions of SON_i, SON_e, and SON_p, (see Section 8) and to determine the rate and extent of degradation of the wastewater samples listed in Table 76. Samples were filtered by Method 1 (Table 56) and placed in acid-washed bottles. Phosphorus (0.2 mg/l P from K_2HPO_4) plus 0.5 ml/liter of one-day-old settled sewage was added as a bacterial seed. The biodegradation bottles with prepared samples were placed in the dark at 20°C and aerated with an air-CO_2 mixture to maintain an aerobic environment, to provide gentle mixing, and to control pH (7.0-7.5). Air and CO_2 were provided in a manner identical to that depicted in Figure 8 except that porous glass diffusers were not used. Sample evaporation was prevented by bubbling the gases through deionized water for humidification.

TABLE 76. LIST OF SAMPLES CHARACTERIZED

Filtered and unfiltered Palo Alto untreated wastewater.
Filtered and unfiltered Palo Alto primary effluent.
Filtered Palo Alto activated-sludge effluent.
Filtered laboratory AS culture effluent prior to steady-state operation.
Filtered laboratory AS culture effluent during steady-state operation.

Samples were taken on Day 0 and at frequent intervals thereafter, filtered, and subjected to the following analysis: SON, SCOD, NH_3-N, NO_2^--N, and NO_3^--N. Filtration was by Method 2 and SON analyses by the Technicon method for Biodegradation Study 3 (Section 8). All other studies used Filtration Method 1 and Kjeldahl SON analysis. Unfiltered organic nitrogen and COD analyses were conducted during Biodegratation Study 3. The pH was monitored frequently during all studies to note the onset of nitrification (significant decrease in pH). As pH decreased, $NaHCO_3$ was added to maintain pH between 7.0 and 7.5.

Molecular Weight Distribution Using Sephadex

Molecular weight distribution was estimated with Sephadex G-15 gel (Pharmacia Fine Chemicals, Piscataway, N.J.). This gel has a molecular weight separation range of 0-1500, and was selected since our preliminary studies and data in the literature [129,209,210,211] indicated that the major fraction of wastewater organics is contained in this range. Data from Sephadex studies helped to determine the nature of activated-sludge effluent SON, and was used to compare the distribution for different SON sources.

Preparatory sample treatment included concentrating with an evaporator concentrator (CALAB, Emeryville, CA). Pall-filtered 500-ml samples were placed in a 1-liter concentration flask and attached to the evaporator-concentrator. The sample flask was partially immersed in a 40°C water bath. Samples were concentrated to approximately 50-100 ml , and if a greater mass of organic material was desired, additional 500-ml aliquots were added and concentrated to give the desired concentration of organics. A precipitate was formed during the concentration step and was removed by a 0.45μ Millipore filter. Recoveries of SON and SCOD were measured.

Sephadex gels were prepared as described in the manual Sephadex: Gel Filtration in Theory and Practice [219]. Gels were allowed to swell in excess eluant on a boiling water bath and were then carefully poured into a K26/100 chromatographic column (Pharmacia Fine Chemicals). Inner diameter of the column was 26 mm, column length was 100 cm, and column capacity was 530 ml. Once the column was filled with swollen gel to the desired volume, a R25/26 eluant reservoir (Pharmacia Fine Chemicals) was attached and several bed volumes of eluant were passed through the column to allow for equilibration of the gel. Void volume was determined by passing high molecular weight Blue Dextran ($MW = 2 \times 10^6$) through the column. Molecular weight estimations were made by comparing elution properties of a series of standard compounds (Table 77) of known molecular weight with those of the unknown sample [219].

Five-ml aliquots of the concentrate were applied to the prepared columns and eluted with 0.04N Na_2SO_4 in deionized water (pH = 7.6-7.8) at flow rates of 0.5-0.7 ml/min. Column effluents were collected in 10-ml aliquots using an automatic fraction collector (SMI 1205, Emeryville, CA). The aliquots were combined to form five larger fractions and duplicate SON (Technicon method) and SCOD analyses were conducted on each fraction.

TABLE 77. COMPOUNDS USED FOR MOLECULAR WEIGHT CALIBRATION OF SEPHADEX COLUMNS

Compound	Molecular Weight
Blue Dextran (Standard Reference Compound)	2×10^6
Poly-DL-Alanine	1,800
Streptomycin sulfate	565
Phenylphenylalanine	312
1-phenylalanine	165

Free and Combined Amino Acid Analysis

Free and combined acid concentrations in Palo Alto activated-sludge effluent were estimated using a Beckman automatic amino acid analyzer made available by the Chemical Evolution Branch of the NASA Ames Research Laboratory in Mountain View, California. The techniques used were developed by personnel at the Ames Laboratory.

Samples for analysis were concentrated by procedures described under Molecular Weight Distribution Using Sephadex. Free amino acids were separated from the concentrated sample by elution with four bed volumes (≈50 ml) of distilled water through a 12-mm-diameter Chromaflex chromatographic column (No. K42251-2512, Kontes Glass Company, Berkeley, CA) packed with about 25 gm of Dowex AG50W-8 cationic-exchange resin in the hydrogen form (Bio-Rad Laboratories, Richmond, CA). Elution of the amino acids from the column was accomplished with four bed volumes of 2N NH_4OH. Flow rates were approximately 0.5 ml/min. Combined amino acids were hydrolyzed in 6N HCl at 110°C for 18 hours, evaporated to dryness, resolubilized in distilled water, pH adjusted to 6.0 with NaOH, and separated as described above. Organic recoveries through those procedures were calculated by monitoring SOC.

After amino acids were eluted, NH_4OH was collected in a 6N HCl trap. Evaporated samples were then analyzed on the Beckman analyzer by trained personnel at the Ames Research Center. The automated technique for amino acid separation and detection was similar to that developed by Spackman, Stein, and Moore [221,222]. Concentrations in the unknown sample were calculated using a chromatogram obtained from a standard amino acid mixture containing known concentrations of amino acids.

RESULTS

Biodegradability

Objectives--

The objective of these studies was to determine the biodegradability of SON contained in different treated and untreated wastewaters. These data would provide information about the relative nature of the SON, the effects of SON on the environment, and the refractory fractions of SON_i, SON_e, and SON_p (Eqs. 1 to 3).

Procedures--

Table 78 lists the wastewaters and treated wastewaters evaluated. Nitrate nitrogen was minitored during biodegradation as concentrations exceeding 10 mg/l interfere with SON analysis.

Decay Rate--

One method of comparing the SON biodegradability of different wastewaters is to compare their decay rates. The reduction in biodegradable SON was expected to follow first-order kinetics [73,207], and a first-order decay rate (k_n, day^{-1}) was calculated from the following after Parkin and McCarty [73]:

$$SON_t = (SON_o - SON_{rf})\ (Ae^{-k_n t}) + SON_{rf} \qquad (27)$$

where SON_t is the SON remaining (mg/l) after t days of degradation, and SON_{rf} is the refractory SON (mg/l). A is a fitting parameter included since the

TABLE 78. WASTEWATERS EVALUATED FOR BIODEGRADABILITY

Biodegradation Study Number	Sample
BS 1	Filtered Palo Alto AS Effluent Filtered Palo Alto Primary Effluent
BS 2	Filtered Palo Alto AS Effluent Filtered and Unfiltered Palo Alto Primary Effluent Filtered and Unfiltered Palo Alto Raw Wastewater
BS 3	Batch Study 1 (SON Removal Studies): Primary-Fed and Control Systems after 0, 6, and 24 Hours Aeration
BS 4	Filtered AS Culture 1 Effluent during Logarithmic Growth of Culture Start-Up
BS 5	Filtered AS Culture 1 and 2 Effluents during Steady-State Operation

Filtration as described in Chapter 4

initial SON measurement, SON_o, is no more accurate than SON measurements at any other time. SON_{rf} was estimated by a trial and error procedure in which an initial value for SON_{rf} was assumed. A and k_n were evaluated using a least-squares experimental curve fit, and a correlation coefficient (r) was calculated. The procedure was repeated until a maximum value of r was obtained for the experimental data and assumed SON_{rf}. It was hoped that this procedure would provide a reasonable estimate for refractory SON and decay rate. Decay rates for SCOD (k_s) were calculated in a similar manner. The percent refractory SON contained in the various wastewater samples was assumed to equal the calculated SON remaining after 60 days degradation using the A and k values obtained as described above. The assumption is somewhat arbitrary, but was felt to be satisfactory for comparative purposes.

Treated and Untreated Wastewaters--

The biodegradability of SON and SCOD contained in activated-sludge effluent, primary and raw wastewaters, and primary wastewater was evaluated. Results are summarized in Tables 79 and 80.

TABLE 79. SUMMARY OF RATE AND EXTENT OF BIODEGRADABILITY FOR TREATED AND UNTREATED WASTEWATERS

Sample (Biodegradation Study No.)	SON			SCOD		
	Percent Refractory[a]	k_n (day^{-1})	r^b	Percent Refractory[a]	k_n (day^{-1})	r^r
Filtered raw wastewater (BS 2)	27	0.075	0.96	27	0.21	0.93
Unfiltered raw wastewater (BS 2)	19	0.16	0.97	26	0.16	0.97
Filtered primary effluent (BS 1)	21	0.15	0.98	24	0.30	0.98
Filtered primary effluent (BS 2)	38	0.12	0.95	29	0.40	0.98
Unfiltered primary effluent (BS 2)	18	0.14	0.97	28	0.24	0.98
Filtered AS effluent (BS 1)	40	0.016	0.82	100	-	-
Filtered AS effluent (BS 2)	50	0.012	0.97	100	-	-

[a] Percent refractory = concentration remaining after 60 days degradation (predicted) divided by original concentration.

[b] r = correlation coefficient for first-order decay model fit.

TABLE 80. BIODEGRADABILITY OF REMAINING SOLUBLE ORGANICS AFTER VARIOUS PERIODS OF TREATMENT OF PRIMARY EFFLUENTS.
Samples Taken during Batch Study 1 (BS 3: Day 0 = 8-18-75)

Day of Incubation	Time 0-Hr. Primary Effluent[a]		Time 0-Hr. Tap Water		Time 6-Hr. Primary Effluent		Time 6-Hr. Tap Water		Time 24-Hr. Primary Effluent		Time 24-Hr. Tap Water	
	SON*	SCOD*	SON	SCOD	SON	SCOD	SON	SCOD	SON	SCOD	SON	SCOD
0	2.37	60	0.35	14	0.90	20	0.41	10	2.68	23	1.17	13
5	1.85	24	0.54	9	1.09	18	0.72	10	2.84	21	1.25	10
10	1.50	23	0.43	11	1.14	19	0.72	10	2.71	20	1.06	12
15	1.48	22	0.61	11	1.26	-	0.71	10	2.68	23	1.11	13
20	1.81	22	0.66	12	1.23	25	0.54	11	2.46	27	1.17	14
30	1.72	-	0.54	14	1.43	-	0.41	12	(2.12)	29	1.07	14

[a] Percent refractory SON = 62 (Day 15); percent refractory SCOD = 36 (Day 15); all other sample SON and SCOD values increased during degradation.

* Values in parentheses represent suspected nitrate interference with Kjeldahl SON analyses.

SON and SCOD in untreated wastewaters were from 18-38 and 24-29 percent refractory, respectively. Treated wastewaters were more refractory, with SON and SCOD being 40-50 and 100 percent refractory, respectively. Fluctuations in the SCOD data prevented obtaining a more reliable estimate for refractory SCOD.

Decay rates for the biodegradable portion of untreated wastewater SON were much higher (0.075-0.17 day^{-1}) than for treated wastewater SON (0.012-0.016 day^{-1}). Untreated wastewater SON and SCOD (0.16-0.40 day^{-1}) decay rates were similar to those reported for BOD in domestic sewage [24]. Variability in SCOD data prevented the calculation of reliable SCOD decay rates for activated-sludge effluent samples.

Examination of rate constants (Table 79) and changes in SON/SCOD ratios during degradation of primary and raw wastewaters shows that SON behaved differently than SCOD; SCOD was in general degraded at a faster rate. This agrees with data presented under SON removal, and emphasizes that the portion of total organics represented by SON acts differently from the organics in general as measured by SCOD.

The SON remaining after 30 days of biodegradation of a partially treated primary effluent was significantly higher than that remaining after 6 hours of high MLSS activated-sludge aeration of the same effluent (Table 80) at a 99 percent level of confidence. This suggests that a short aeration time with high MLSS activated-sludge treatment may give a better estimate of untreated wastewater refractory SON (SON_r, Eq. 1) than long-term, microbial seed degradation.

SON and SCOD released during organism decay was not degradable (Table 80, Tap Water Control Systems). SON and SCOD in general increased during degradation, perhaps due to production by the microbial seed from oxidation of utilizable organics in the sample, and/or from seed organism decay. Seed concentrations were the same (0.5 ml/l of day-old settled sewage) as for other Biodegradation Studies, and seem too low for seed organism decay to entirely explain the increase in SON and SCOD. Regardless, data from the Time 0 control sample indicates that the "equilibrium level" SON (SON_{eq}) released upon dilution of activated sludge is refractory, an important observation because it implies that SON_{eq} will not be removed during treatment and may persist in the environment upon discharge.

Biologically Produced Organics--

The biodegradability of SON produced during start-up and during steady-state operation of AS Cultures was evaluated (Table 81). For BS 4, a composite of AS Culture 2 effluent from three consecutive days (Days 15-17) was taken near the time when the start-up peak concentration of SON occurred (Figure 17). For BS 5, samples were taken during steady-state operation, on Days 302-304 for AS Culture 1 and Days 141-143 for Culture 2. Samples for the first two days were filtered and stored at 4°C prior to combining with effluent from the third day.

The SON produced near the peak concentration during start-up was 20 percent refractory, a value lower than for treated wastewater SON, but nearly

TABLE 81. SUMMARY OF RATE AND EXTENT OF BIODEGRADABILITY FOR BIOLOGICALLY PRODUCED ORGANICS

Sample (Biodegradation Study No.)	SON			SCOD		
	Percent Refrac. tory[a]	k_n (day^{-1})	r[b]	Percent Refractory	k_s (day^{-1})	r
Filtered AS Culture 2 during start-up (BS 4)	20	0.027	0.94	40	0.075	0.87
Filtered AS Culture 1 during steady-state (BS 5)	18	0.029	0.94	31	0.14	0.95
Filtered AS Culture 2 during steady-state (BS 5)	100	-	-	39	0.090	0.90

[a] Percent refractory = concentration remaining after 60 days degradation (predicted) divided by initial concentration.

[b] r = correlation coefficient for first-order decay model fit.

equal that for untreated wastewater SON (Table 79). SON decay rates were low (about 0.028 day^{-1}), about the same as those reported in Table 79 for activated-sludge effluent samples. SCOD decay rates were somewhat higher than SON decay rates.

There was a significant difference in the degradability of SON produced during steady-state operation by AS Cultures 1 and 2: AS Culture 2 SON was 100 percent refractory while AS Culture 1 was about 20 percent refractory. The initial SON for AS Culture was significantly higher (0.76 mg/l) than that of Culture 2 (0.24 mg/l), and Culture 1 had undergone a noticeable change in cultural characteristics 10 days prior to taking samples for evaluation. During this cultural change, MLSS dropped from around 2000 mg/l to 1600 mg/l and SON increased from about 0.4 mg/l to near 0.8 mg/l. Culture 2 exhibited stable operation (no cultural changes) for more than 20 days prior to sample testing. These differences in cultural history provide a partial explanation for differences in SON degradability, since throughout this report, cultural characteristics have been linked to effluent characteristics and behavior. SCOD degradabilities and decay rates were similar for the two cultures.

Changes in SON/SCOD ratios during degradation of steady-state AS Culture 1 and 2 samples again emphasize that the SON subset of organics acts differently from SCOD in general. The effect was most pronounced for AS Culture 2, as SON remained constant while SCOD decreased. Significantly more degradable

SON was produced during start-up of AS Culture 2 (1.63 mg/l) than during steady-state operation (0 mg/l). A higher concentration of refractory SON (0.4 mg/l) was produced during initial start-up than during steady-state operation (0.26 mg/l); differences were significant at a 99 percent level of confidence, but were not great.

Summary--

Biodegradation studies have given estimates of the refractory fractions of SON_i (untreated domestic wastewaters), SON_e (activated-sludge treated domestic wastewaters), and SON_p (SON produced by AS Cultures). SON_i is 18 to 38 percent refractory, with the biodegradable portion degrading at rates of 0.075-0.17 day^{-1}. SON_e was from 40 to 50 percent refractory; decay rates were 0.012 to 0.016 day^{-1}, values much less than those for untreated wastewaters. SON produced biologically by AS Cultures was 18 to 100 percent refractory, depending on culture characteristics, and decayed at rates of about 0.030 day^{-1}.

SON released during organism decay was refractory, as was SON_{eq}. SON and SCOD exhibited different degradabilities; SCOD was degraded at a faster rate. Cultural characteristic changes affected the quantity of refractory SON produced by activated-sludge treatment of a low SON synthetic waste. More degradable SON was produced during AS Culture start-up than during steady-state operation.

Molecular Weight Distribution

Objective--

The objective of these experiments was to determine the molecular weight distribution, as defined by Sephadex gel filtration, for selected wastewaters. This information was used to compare molecular weight characteristics of different SON sources and to give clues as to the nature of the soluble nitrogen-containing organics.

Procedures--

Samples analyzed included filtered Palo Alto raw waste-water, primary and AS effluents and AS Culture 2 effluent taken near peak SON concentration during culture start-up. The raw, primary, and AS Culture 2 samples were the same as evaluated in Biodegradation Studies 2 and 4, respectively.

Concentrated samples were eluted through Sephadex G-15 gel which has a nominal molecular weight exclusion limit of 1500. Eluted fractions were combined in the following molecular weight (MW) ranges: < 165, 165-340, 340-780, 780-1800, and > 1800, as defined by passing organic compounds of known molecular weight (Table 77) through the column. A set of molecular weight standards was eluted before and after each set of wastewater samples. Elution patterns were identical for all sets of standards checked.

For the raw wastewater and primary effluent samples, replicate 5-ml aliquots were eluted to check reproducibility of the elution procedure. Duplicate SCOD and Technicon SON analyses were run for each MW fraction. The SON and SCOD of sample-free eluant were also determined.

Statistical methods used to evaluate experimental data are presented in Appendix A under Statistical Methods Used for Molecular Weight Distribution Studies.

Sample Concentration--

Table 82 contains a summary of SON and SCOD recoveries obtained during sample concentration and filtration. Recoveries during concentration were 79 ± 9 percent for SON and 66 ± 9 percent for SCOD. Losses were associated with a precipitate formed during concentration and removed by filtration with a 0.45μ Millipore filter.

During the vacuum concentration step, an average of 90 percent of the NH_3-N was lost from the samples, reducing potential interference in the SON analysis.

Molecular Weight Distribution Results--

Tables 83 and 84 respectively summarize SON and SCOD data obtained from Sephadex separation. All SON and SCOD mass values listed are averages of duplicate analyses. Experimental uncertainties indicated represent values for a 95 percent level of confidence.

Reproducibility of the elution procedure was very good as indicated by results for replicate elutions of primary effluent and raw wastewater (Tables 83 and 84). SON recoveries through the column were 86 ± 8 percent for the 6 samples tested, and this represented 70 ± 4 percent of the SON in the original concentrated sample. Corresponding SCOD recoveries were 92 ± 11 percent and 56 ± 11 percent.

Some caution must be exercised when interpreting results from these MW experiments since experimental uncertainty was fairly high, and potential changes in molecular characteristics, and hence molecular weight distribution, caused by the concentration procedure are unknown. However, trends can be noted, and in certain instances, specific conclusions about the MW distributions can be made.

Molecular weight distributions for the four samples studies, expressed as percent of SON or SCOD present in the original sample, are summarized in Figures 34 to 37. Of the material recovered and measured by gel filtration, 50 to 60 percent of the SON and SCOD in raw, primary, and activated-sludge samples had molecular weights less than 1800. These results agree with data presented by others [28,129,209,210,211]. MW distributions for filtered raw, primary effluent, and activated-sludge effluents were very similar. The activated-sludge effluent contained a slightly higher > 1800 MW fraction and slightly lower 165-340 MW fraction than did other samples, but differences were not statistically significant at a 95 percent confidence level. These data contradict results by Zuckerman and Molof [211] who reported that activated-sludge treatment selectively removes the 400 MW fraction, leaving behind the 1200 MW fraction.

The MW distribution pattern for AS Culture 2 effluent during start-up, however, differed markedly from the wastewater samples just described. Twenty-three percent of the SON and 41 percent of the SCOD had measured

TABLE 82. SUMMARY OF SON AND SCOD RECOVERIES FROM CONCENTRATION AND FILTRATION

Sample	Volume Concentrated (liter)	SON Mass (mg)			Percent SON Recovery	SCOD Mass (mg)		Percent SCOD Recovery
		Concentration Factor	Before Concentration	After Concentration & Filtration		Before Concentration	After Concentration & Filtration	
Secondary Effluent	1.71	46	1.74 ± 0.07	1.21 ± 0.05	70	37.0 ± 2.1	28.4 ± 1.5	77
Secondary Effluent	1.71	17	1.74 ± 0.07	1.43 ± 0.05	82	37.0 ± 2.1	29.2 ± 1.5	79
Secondary Effluent[a]	1.0	28	1.16 ± 0.03	1.00 ± 0.04	86	20.6 ± 0.8	13.4 ± 0.2	65
Secondary Effluent[b]	2.0	47	2.22 ± 0.06	1.56 ± 0.07	70	47.2 ± 1.6	27.6 ± 1.8	58
Primary Effluent[b]	1.0	20	5.50 ± 0.17	4.66 ± 0.19	85	92.7 ± 2.3	50.8 ± 0.4	55
Raw Wastewater[b]	1.0	20	5.31 ± 0.20	4.78 ± 0.15	90	89.5 ± 2.9	53.5 ± 4.9	60
AS Culture 2 Effluent[b]	2.0	36	4.06 ± 0.08	2.88 ± 0.10	71	89.0 ± 1.4	63.4 ± 1.0	71

[a] Sample used to check concentration recoveries. SON and SCOD mass values are averages ± standard deviations of replicate samples.

[b] Samples used for molecular weight characterization; SON and SCOD mass values are averages ± standard deviations of replicate samples.

TABLE 83. SUMMARY OF SON ELUTION DATA FOR MOLECULAR WEIGHT DISTRIBUTION STUDIES

	μg SON Added to Column	μg SON Recovered in each Molecular Weight Range*					Percent Recovery through Column	Percent Recovery through Column
		< 165	165-340	340-780	780-1800	>1800		
Raw wastewater:								
Elution 1	467 ± 23	11.6 ± 23.1	189.3 ± 14.5	120.4 ± 14.4	81.5 ± 14.4	18.1 ± 14.4	90 ± 0	77 ± 7
Elution 2	467 ± 23	8.7 ± 23.1	185.1 ± 14.5	102.9 ± 14.3	72.6 ± 14.5	10.0 ± 14.5	81 ± 9	69 ± 7
Primary Effluent:								
Elution 1	478 ± 22	24.8 ± 23.1	149.6 ± 14.6	95.5 ± 14.6	81.8 ± 14.8	15.6 ± 14.6	77 ± 9	69 ± 8
Elution 2	478 ± 22	27.7 ± 23.1	158.5 ± 14.6	107.9 ± 14.7	77.8 ± 14.7	17.4 ± 14.6	79 ± 9	71 ± 8
AS Effluent	178 ± 10	16.3 ± 23.2	59.4 ± 14.8	47.9 ± 14.7	28.4 ± 14.6	19.2 ± 14.6	92 ± 22	67 ± 15
AS Culture 2 during start-up phase	262 ± 16	7.7 ± 24.6	54.8 ± 15.6	17.6 ± 15.6	75.6 ± 14.7	85.6 ± 14.7	92 ± 16	65 ± 11

*± values represent 95 percent confidence limits for analyses.

TABLE 84. SUMMARY OF SCOD ELUTION DATA FOR MOLECULAR WEIGHT DISTRIBUTION STUDIES

Sample	mg SCOD Added to Column	mg SCOD Recovered in each Molecular Weight Range*					Percent Recovery through Column	Percent Recovery through Column
		< 165	165-340	340-780	780-1800	>1800		
Raw Wastewater:								
Elution 1	5.08 ± 0.41	0.22 ± 0.24	0.43 ± 0.15	1.86 ± 0.15	1.21 ± 0.15	0.52 ± 0.15	83 ± 10	46 ± 5
Elution 2	5.08 ± 0.41	0.26 ± 0.24	0.43 ± 0.15	1.83 ± 0.15	1.31 ± 0.15	0.47 ± 0.15	83 ± 10	46 ± 5
Primary Effluent:								
Elution 1	5.35 ± 0.40	0.44 ± 0.24	0.46 ± 0.15	2.21 ± 0.15	1.15 ± 0.15	0.44 ± 0.15	83 ± 10	53 ± 5
Elution 2	5.35 ± 0.40	0.33 ± 0.24	0.51 ± 0.15	2.19 ± 0.15	1.03 ± 0.15	0.38 ± 0.15	83 ± 10	53 ± 5
AS Effluent	3.14 ± 0.27	0.34 ± 0.25	0.36 ± 0.15	1.19 ± 0.15	0.88 ± 0.15	0.48 ± 0.15	104 ± 15	60 ± 7
AS Culture 2 during start-up phase	5.76 ± 0.34	0.15 ± 0.26	0.18 ± 0.16	0.93 ± 0.16	1.57 ± 0.15	3.32 ± 0.15	107 ± 9	76 ± 6

*± values represent 95 percent confidence limits for analyses.

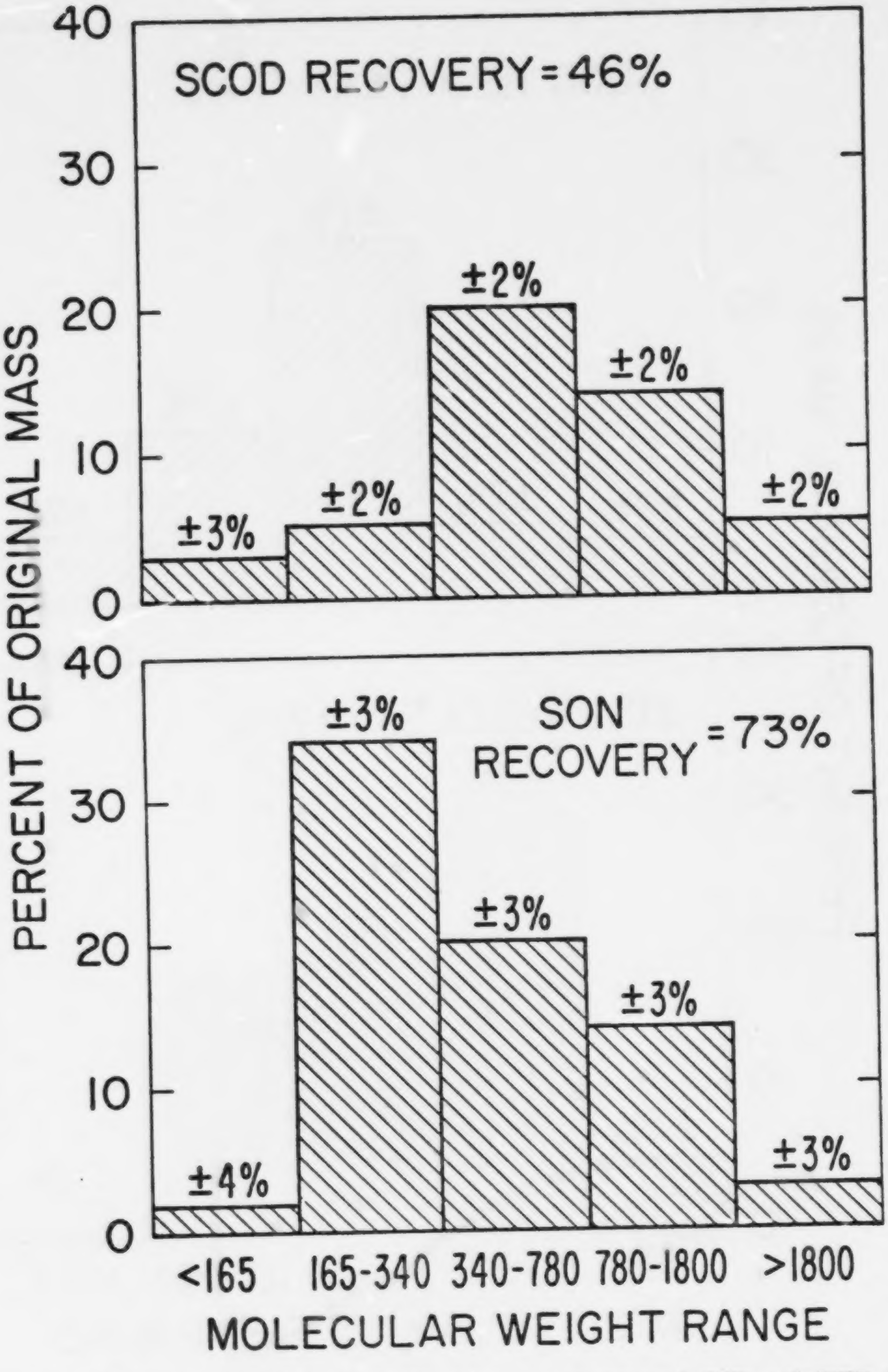


Figure 34. Molecular weight distribution of recovered SON and SCOD for soluble raw wastewater as percent of mass in original sample. ± figures are 95 percent confidence limits.

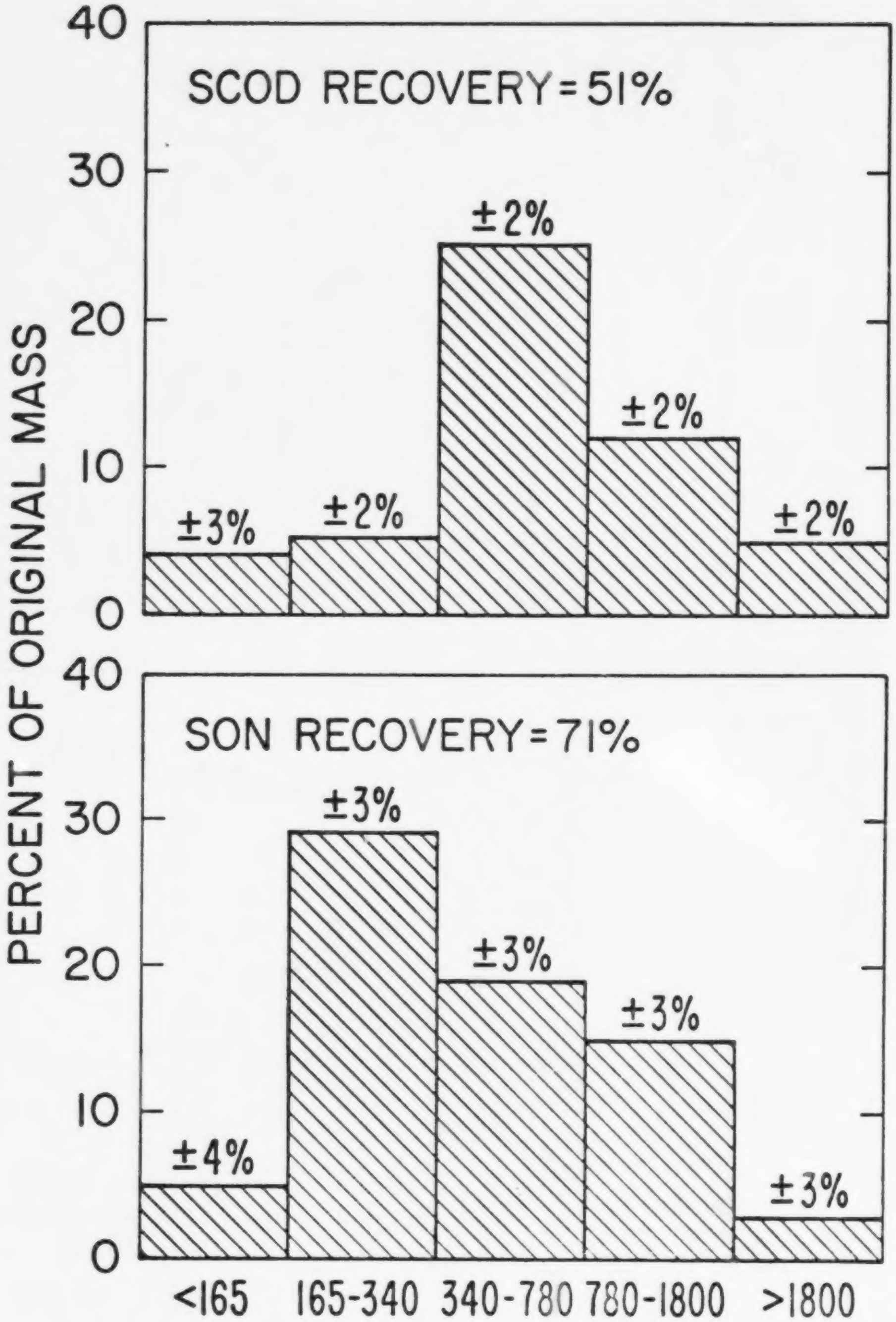


Figure 35. Molecular weight distribution of recovered SON and SCOD for soluble primary effluent as percent of mass in original sample. ± figures are 95 percent confidence limits.

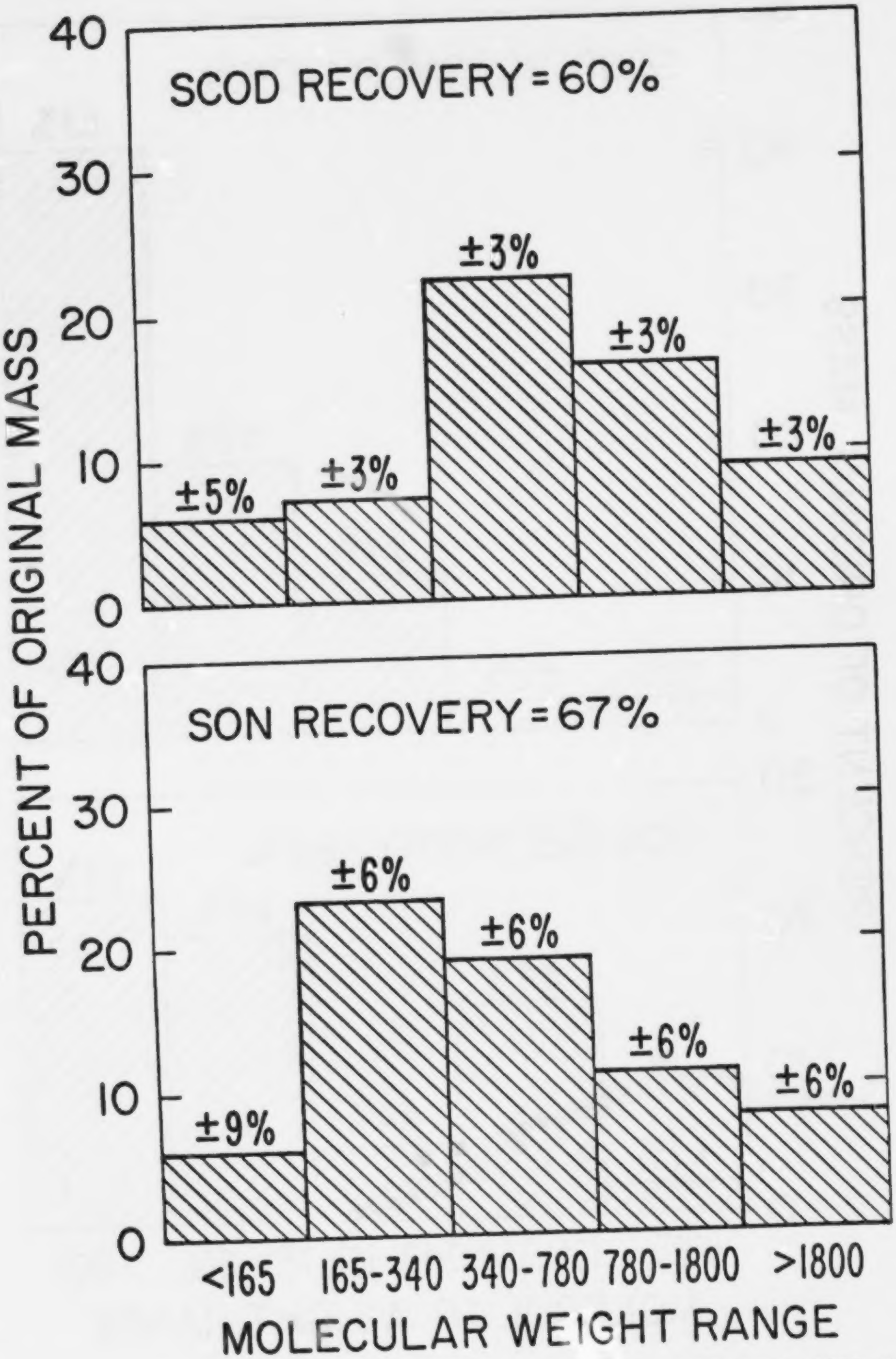


Figure 36. Molecular weight distribution of recovered SON and SCOD for soluble activated-sludge effluent as percent of mass in original sample. ± figures are 95 percent confidence limits.

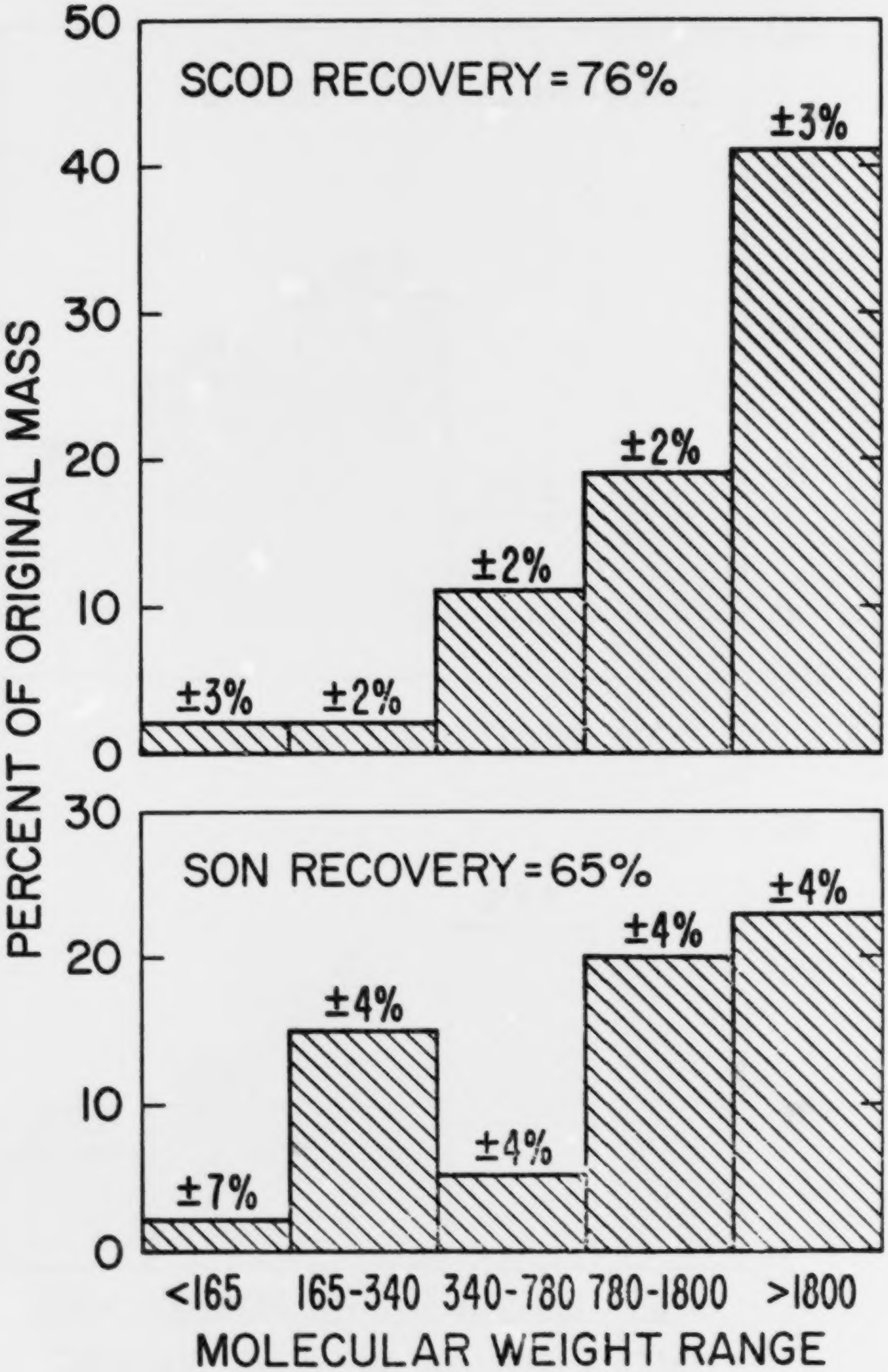


Figure 37. Molecular weight distribution of recovered SON and SCOD for soluble AS Culture 2 effluent during start-up as percent of mass in original sample. ± figures are 95 percent confidence limits.

molecular weights greater than 1800. During culture start-up, bacteria produce and excrete high molecular weight compounds such as RNA, enzymes, and proteins, partially explaining the increased proportion of higher MW, SON and SCOD compounds observed [32,101,113,148].

There is an apparent difference in the nature of the materials in the various MW fractions as indicated by the SON/SCOD ratios (Table 85). Uncertainties (95 percent confidence limits) were very high for ratios in the < 165 and 165-340 ranges due to the low SCOD values. The ratios for all MW fractions except the 165-340 range were very similar, averaging 0.045 ± 0.015. SON/SCOD values for the 165-340 fraction, however, were significantly higher (95 percent confidence level), ranging from 0.165 to 0.435 for the 4 samples studied, and averaging 0.306 ± 0.111.

Theoretical SON/SCOD ratios for typical nitrogen-containing organics of varying molecular weights are listed in Table 86. Nucleic acid bases, their derivatives, and other heterocyclic nitrogen compounds are the most likely candidates for the 165-340 MW fraction. Creatinine and hypoxanthine are examples of these compounds found in wastewaters and treated wastewaters [5]. Most other classes of nitrogen-containing organics have similar SON/SCOD ratios.

An explanation for the high SON/SCOD ratio of the 165-340 MW fraction might be that the organics present are resistant to oxidation by the COD procedure, thus resulting in high measured SON/SCOD ratios. Organics known to be incompletely oxidized include pyridine and its derivatives, aromatic hydrocarbons, methylamines, and ethylamines [3,140]. Purine, pyrimidines, pyroles, and their derivatives are completely oxidized [140]. The former group of compounds are not likely to be present in large quantities in domestic wastewater nor produced during biological treatment, while the latter group of SON compounds are known to be produced biologically and to be present in treated and untreated wastewaters [5,32,101,148]. Therefore, it seems more reasonable that the high SON/SCOD ratio for the 165-340 MW fraction was caused by the presence of nucleic acid bases and/or other heterocyclic nitrogen compounds.

Comparison of SON/SCOD ratios for primary and activated-sludge effluents showed that significantly more SCOD than SON was removed from the 165-340 and 780-1800 MW fractions during activated-sludge treatment (95 percent confidence level).

Summary--

Molecular weight distribution studies using Sephadex gel filtration has provided useful information about the nature and source of SON. At least 50 to 60 percent of the SON and SCOD in filtered raw wastewater, primary effluent, and activated-sludge effluent represented small molecules having molecular weights of less than 1800. Only three to nine percent of the measured organics were contained in the > 1800 fraction. However, approximately 30 and 50 percent of the SON and SCOD, respectively, were not recovered and hence were not measured by the Sephadex procedure. The MW distribution of this fraction in unknown.

TABLE 85. SON/SCOD RATIOS FOR THE MOLECULAR WEIGHT FRACTIONS OF THE FILTERED WASTEWATERS STUDIED

Sample	Molecular Weight Range*				
	< 165	165-340	340-780	780-1800	> 1800
Primary Effluent	0.068 ±0.052	0.318 ±0.071	0.046 ±0.005	0.073 ±0.012	0.040 ±0.027
Raw Wastewater	0.042 ±0.073	0.435 ±0.178	0.061 ±0.006	0.061 ±0.009	0.028 ±0.021
Activated-Sludge Effluent	0.048 ±0.077	0.165 ±0.079	0.040 ±0.013	0.032 ±0.017	0.040 ±0.024
AS Culture 2 Effluent during start-up phase	0.051 ±0.177	0.304 ±0.300	0.020 ±0.017	0.048 ±0.010	0.026 ±0.011

*± values are 95 percent confidence limits for SON/SCOD ratios (Appendix B, item 9).

TABLE 86. THEORETICAL SON/SCOD FOR TYPICAL NITROGEN-CONTAINING ORGANIC COMPOUNDS OF DIFFERING MOLECULAR WEIGHTS

MW Range	Class of Compound	Theoretical SON/SCOD ratios
< 165	Amino acids	0.02-0.15
	Urea	∞
	Small nucleic acid bases	0.20-0.70
165-340	Nucleic acid bases	0.20-0.40
	Heterocyclic nitrogen compounds	0.20-0.40
	Small peptides	0.05-0.15
	Small nucleotides and nucleosides	0.10-0.20
	Amino sugars	< 0.10
	Hydroxaminic acids	0.05-0.15
340-780	Peptides and amines	0.05-0.15
	Small proteins	0.05-0.15
	Ionophores	0.05-0.15
	Nucleotides and nucleosides	0.10-0.15
780-1800	Proteins and amines	0.05-0.15
	Ionophores	0.05-0.15
	Nucleotides and nucleosides	0.10-0.15
	Coenzymes	0.05-0.15
	Vitamins	0.05-0.15
> 1800	Large proteins	0.05-0.10
	Enzymes	0.05-0.10
	RNA	0.10-0.15
	DNA	0.10-0.15
	Humic acids	< 0.10

MW distributions for the three wastewaters from various stages of treatment were not significantly different. However, activated-sludge treatment did remove significantly more SCOD than SON from the 165-340 and 780-1800 MW fractions. SON and SCOD produced during start-up of AS Culture 2 exhibited a significantly different MW distribution, with 24 and 41 percent of the SON and SCOD, respectively, contained in the > 1800 fraction.

The nature of the organic material in the 165-340 MW range with an average SON/SCOD ratio of 0.306, was significantly higher than that of all other MW ranges (average SON/SCOD - 0.045). This high SON/SCOD ratio suggests that heterocyclic nitrogen compounds and nucleic acid bases are the major constituents in the 165-340 MW fraction.

Free and Combined Amino Acids

Analysis of free and combined amino acids was conducted to estimate the contribution of these constituents to activated-sludge effluent SON. Filtered Palo Alto activated-sludge effluent was analyzed and results are summarized in Table 87.

Amino acid recovery was not determined directly, but was estimated by monitoring soluble organic carbon (SOC) recovery from each step of the procedure. Recoveries for the free and combined amino acid procedures were 50 and 25 percent, respectively. Calculating amino acid recoveries using SOC recovery data may yield high estimates for amino acid concentrations since the desalting procedure used was designed to select for amino compounds, and this should give higher SON than SOC recoveries. An additional potential error is introduced by the concentration procedure in which SON and SOC recoveries were 70 and 75 percent, respectively. Any amino acids lost during the concentration step would not be measured. Even with these limitations, observed data used in conjunction with supporting literature data provided useful information about activated-sludge effluent SON.

A relatively small fraction of activated-sludge effluent SON and SOC was comprised of free and combined amino acids (Table 87). These values are within the percentages of SOC for free and combined amino acids of 0.1 to 4.6 and 1.7 to 10 percent, respectively, reported by others [5,23,213,223,224]. Although none of these previous reports indicate amino acid concentration as a fraction of SON, it can be stated with reasonable assurance that these materials comprise a relatively small fraction of activated-sludge effluent SON.

DISCUSSION OF RESULTS

Summary of Experimental Results

Biodegradability--

Table 88 contains a summary of percent refractory SON and SCOD and decay rate data for the different types of wastewaters studied.

Treated wastewaters were much more refractory than untreated wastewaters. SON decay rates for the treated wastewater were very low, as were SON decay rates for SON produced by laboratory AS Cultures. SCOD in general degraded at a faster rate than SON, emphasizing differences in the nature of the nitrogen-containing organics and the general organic fraction as measured by SCOD.

An important observation is the variability in the degradability of the two AS Cultures operating under steady-state conditions. Cultures had similar refractory SON concentrations, 0.14 and 0.26 mg/l for AS Cultures 1 and 2, respectively, but AS Culture 1 contained 0.62 mg/l biodegradable SON while AS Culture 2 contained no degradable SON.

TABLE 87. AMINO ACID DATA FOR PALO ALTO ACTIVATED-SLUDGE EFFLUENT (6-14-74)

Amino Acid	Free Amino Acids		Combined Amino Acids		Total Amino Acids	
	μg/1 as SON	μg/1 as SOC	μg/1 as SON	μg/1 as SOC	μg/1 as SON	μg/1 as SOC
Aspartic Acid	1.2	4.1	2.3	7.9	3.5	12.0
Threonine	1.3	4.5	0.8	2.7	2.1	7.2
Serine	5.5	14.1	-	-	5.5	14.1
Proline	-	-	-	-	-	-
Glutamic Acid	0.7	3.0	3.3	14.1	4.0	17.1
Glycine	3.5	6.0	5.7	9.8	9.2	15.8
Alanine	1.6	4.1	2.3	5.9	3.9	10.0
Valine	-	-	1.5	6.4	1.5	6.4
Methionine	-	-	0.2	0.9	0.2	0.9
Isoleucine	0.4	2.1	-	-	0.4	2.1
Leucine	0.3	1.5	-	-	0.3	1.5
Tyrocine	0.2	1.5	2.3	17.7	2.5	19.2
Phenylalanine	0.3	2.3	0.2	1.5	0.5	3.8
Lysine	4.4	11.3	0.5	1.3	4.9	12.6
Histidine	3.1	5.3	0.2	0.3	3.3	5.6
Total	22.5	59.8	19.3	68.5	41.8	128.3
Percent of Original Effluent SON* or SOC*	3.1	0.9	5.3	2.2	8.4	3.1

*Corrected for estimated recoveries through the analytical procedure (see text).

SON = 1.45 mg/1; SOC = 12.5 mg/1.

TABLE 88. SUMMARY OF BIODEGRADABILITY DATA

Sample	SON		SCOD	
	Percent Refractory	Decay Rate (day^{-1})	Percent Refractory	Decay Rate (day^{-1})
Untreated wastewaters	18-38	0.075-0.16	24-29	0.16-0.40
AS treated wastewaters	40-100	0.014-0.016	100	-
AS Culture 2 during start-up	20	0.027	40	0.075
AS Cultures during steady-state	20-100	0.029	39-40	0.090-0.14

The low-seed microbial procedure for determining biodegradabilities has limitations because it is not known how much refractory SON is produced during the procedure. Results from Biodegradation Study 3 showed that high MLSS batch aeration gave a significantly lower estimate for refractory SON concentration (0.90 mg/l) than did the low microbial seed procedure (1.48 mg/l). Refractory SON may have been produced during the low microbial seed procedure. Regardless of these limitations, and since experimental procedures and methods for data analysis were identical for all biodegradation studies, the percent refractory and decay rate values listed in Table 88 were considered acceptable for comparison of waste-water degradabilities.

Chemical Nature--

Molecular weight distribution studies using Sephadex gel filtration showed that from 50 to 60 percent of the SON and SCOD in treated and untreated wastewaters had apparent molecular weights less than 1800. Three to nine percent had apparent MW greater than 1800. Thirty percent of the SON and 50 percent of the SCOD were not recovered during the experimental procedure and hence the MW distribution of this material was not determined. SON produced during biological oxidation of a glucose-acetate substrate (AS Culture 2) start-up was significantly different in nature from the treated and untreated wastewaters; 24 percent of the produced SON and 41 percent of the SCOD had molecular weights in excess of 1800.

The nature of the SON in the 165 to 340 MW range (containing 15-34 percent of the SON) was significantly different than that in all other MW fractions. The high SON/SCOD ratio of the 165-340 MW fraction (0.306 versus 0.045 for all other fractions) suggested the presence of heterocyclic nitrogen compounds such as nucleic acid bases.

Analysis for free and combined amino acids (proteins) showed that these materials probably comprise less than 10 percent (3.1 and 5.3 percent, respectively) of the SON contained in activated-sludge effluent. This observation agrees well with data available in the literature [5,23,212,223,224].

Implications

Biodegradability--

Organic compounds are refractory for many reasons (Section 8). One of the major reasons for the refractory nature of activated-sludge effluent SON (0 to 60 percent degradable; decay rates of about 0.014 day^{-1}) may be the low concentration of individual SON compounds. Molecular weight distribution studies showed that SON compounds of wide-ranging molecular weights were present; the major fraction, the 165-340 MW range, contained approximately 20 percent of the total SON. If all the SON were a single compound, the concentration would be about 0.2 to 0.3 mg/l, a level which may approach a limit for supporting growth and providing maintenance energy. It is not likely that a single SON compound represents the SON in any MW fraction [5,13,210,224,226]. Degradation of this wide range of SON compounds may thus proceed at very slow rates, if at all. Support for this concentration effect was given by Chudoba [62], who showed that concentration of activated-sludge effluents resulted in additional organic removal (measured as SCOD).

The low decay rates for produced SON (0.027 and 0.029 day^{-1}), and low biodegradability (100 percent in some cases), indicates that this SON will persist for long periods of time upon discharge to receiving waters. The refractory SON is not likely to support algal growth [73,227,228], but the slow release of NH_3-N from the biodegradable portion will provide some nitrogen for growth stimulation.

The biodegradable fraction of activated-sludge effluent SON was found to be variable, ranging from 0 to 60 percent, 0.010-0.045 day^{-1}. The degradable fraction of produced SON ranged from 0 to 80 percent, and was related to changes in culture characteristics (Biodegradation Studies 4 and 5).

The SON in untreated wastewaters was highly degradable, probably due to the presence of readily degradable substances such as urea and free and combined amino acids, the major components of untreated wastewaters [223,229, 230,231]. Activated-sludge treatment efficiently removes these materials [223,224]; in this research, free and combined amino acids represented less than 10 percent of activated-sludge effluent SON.

Chemical Nature--

Both MW distribution studies and earlier reported studies (Section 7) with cationic exchange at pH 2 suggested the presence of nucleic acid bases or heterocyclic nitrogen compounds. Similar cation-exchange studies were conducted on samples analyzed for MW distribution. A comparison of the results of these studies was made to estimate the fraction of SON likely to be comprised of these types of materials. Table 89 summarizes the results of this comparison

Based on apparent molecular weight data nucleic acid bases and heterocyclic nitrogen compounds may comprise up to 23 ± 6 percent of activated-sludge effluent SON. The difference between the SON removed by cationic

TABLE 89. COMPARISON OF RESULTS FROM MW DISTRIBUTION AND CATIONIC-EXCHANGE STUDIES

	Raw Waste-water	Primary Effluent	Activated-Sludge Effluent	AS Culture 2 during start-up	AS Culture 1 during steady-state	AS Culture 2 during steady-state
Percent SON in 165-340 MW Fraction	34 ± 3	29 ± 3	23 ± 6	15 ± 4	-	-
Percent SON removed by cationic resins at pH 2.0	-	50 ± 7	35 ± 7	33 ± 9	56 ± 38	42 ± 12

± values are 95 percent confidence limits.

resins at pH 2 (35 ± 7 percent) and that contained in the 165-340 MW range may be explained by the presence of free and combined amino acids or the presence of nucleic acid degradation products (MW > 165-340). Free and combined amino acids were found to account for approximately 10 percent of Palo Alto activated-sludge effluent SON. Combining MW distribution, ion exchange, and amino acid data suggests that a combination of free and combined amino acids (proteins), nucleic acid degradation products, nucleic acid bases, and heterocyclic nitrogen compounds may account for up to 35 percent of activated-sludge effluent SON.

Heterocyclic nitrogen and nucleic acid bases may comprise up to 30 percent of the SON in untreated wastewaters. Free and combined amino acids or nucleic acid breakdown products may account for the additional 20 percent removed by cationic exchange at pH 2.0. Hanson and Lee [229] found that an average of 36 percent of untreated wastewater SON was free and combined amino acids. They also estimated the uncharacterized SON fraction at 40-50 percent; some of this uncharacterized fraction was most likely material similar to nucleic acid degradation products.

Fifteen percent of the SON produced from oxidation of a glucose-acetate substrate may be comprised of nucleic acid bases and heterocyclic nitrogen compounds. Whether the additional 18 percent SON removal observed with pH 2.0 cationic exchange was due to removal of amino acids and degradation products of nucleic acids is open to speculation.

The presence of significant quantities of nucleic acid degradation products, nucleic acid bases, and heterocyclic nitrogen compounds might have been expected based on reports in the literature [100,101,109,112,148]. Organisms excrete and partially degrade RNA during logarithmic growth and during starvation. RNA degradation products may accumulate to varying degrees while proteins generally do not [112].

The precise chemical nature of SON contained in other MW fractions could not be determined. Possible constituents were listed in Table 86. A popular "catch-all" category for unidentified organic material is humic substances [22,23,26,224]; from 30 to 65 percent of the organic matter in biological treatment plant effluents may be contained in this fraction. Analysis for percent SON associated with such humic substances was not conducted in this research because of the ambiguous nature of this classification method.

SECTION 10

SUMMARY AND DISCUSSION

SON IN MUNICIPAL SECONDARY EFFLUENTS

Analysis of secondary effluents from four activated-sludge plants treating municipal wastewaters indicated that the soluble organic nitrogen (SON) concentrations varied from 1.1 to 2.1 mg/l, with an average of 1.5 mg/l. Analysis of twenty 24-hour composite samples from one treatment plant over a six-month period indicated a range in SON concentration from 0.8 mg/l to 1.7 mg/l, with an average of 1.3 mg/l. The standard deviation was 0.24 mg/l. Thus, the range in concentrations of effluent SON between plants and within plants appears quite small.

This same survey indicated that soluble chemical oxygen demand (SCOD) of the secondary effluents was somewhat proportional to the SON. Ratios of SON to SCOD for the three treatment plants ranged from 0.031 to 0.067 with an average of 0.047.

SON CHARACTERISTICS

A detailed study of the biological removal and production of SON at one treatment plant, together with laboratory studies with synthetic wastewaters indicated that about one-third of the SON in secondary effluents in produced biologically during treatment, and the other two-thirds represents the remainder of SON originally present in the untreated wastewater. The latter material is composed mainly of refractory organic materials which are not degraded after an additional 30 to 60 days of incubation with a bacterial seed. About one-half of the portion of SON formed biologically during treatment represents exudate or cellular material released during organism decay. The other half is released from the cells when they are diluted with new wastewater. This latter portion of SON rapidly establishes an "equilibrium" concentration between the cells and the surrounding fluid which is a function of the bacterial cultural characteristics, and independent of the nature of the water in which they are diluted.

The concentration of biologically produced SON is highly dependent upon the characteristics of the microorganisms comprising activated-sludge. However, for a given culture of microorganisms the extent of production is consistent. In all cases, the concentration of biologically produced SON increases significantly up to several mg/l if there is a disturbance in the system and during start-up of an activated-sludge system. The SON produced during this period appears to be quite readily biodegradable. However, the biologically produced SON resulting during normal treatment plant operation near steady-state is quite resistant to biodegradation.

The SON in untreated wastewater and secondary effluents was characterized in various ways in order to evaluate its nature and source. An evaluation of the distribution of molecular weights of the materials comprising SON indicated that about one-third of the SON organics in secondary effluent had a molecular weight of less than 340, while only about one-eighth of the SCOD was in this molecular weight fraction. Most of the SON and SCOD had molecular weights of less than 1800. However, 30 to 40 percent of the SON and SCOD were not recovered by the analytical procedure. This portion may have been dominated by materials with higher molecular weights. Molecular weight distributions were similar with untreated and primary treated wastewaters, except that the quantities of SON in the molecular weight range below 340 were greater.

The molecular weight distribution of the relatively large quantity of soluble organic materials produced biologically during culture start-up gave a different picture than the above. Here, the fraction of SON with molecular weight less than 340 was only about 20 percent of the total, and the SCOD only about 5 percent of the total.

The molecular weight of almost half of the SON was greater than 780, and of almost half of the SCOD greater than 1800. Thus, in addition to being highly biodegradable, the material produced during culture start-up was comprised of larger molecules.

Various lines of evidence suggest that about one-third of the SON, in particular that with molecular weight less than 340, is comprised of nucleic acid degradation products. Other studies with pure cultures indicate excretion of such products is common among bacteria. The low COD of this material is consistent with this hypothesis. In addition, the ability to remove a significant fraction of the SON by cation exchange at low pH also suggests the presence of such products. It would be worth while to direct some future studies specifically towards measurement for such degradation products.

Analysis specifically for amino acids and proteins indicated that they comprised less than 10 percent of the SON in secondary treatment plant effluents. This finding is similar to that reported by others.

About 50 percent of the SON could not be characterized by the analytical procedures used. However, through use of physical and chemical techniques, the materials could be operationally classified in a manner which was useful for predicting removal of these materials by physical and chemical processes. Figure 7, contained in Section 7, presents this fractionation in pictorial form. About 10 percent of the SON appears to be dominated by a positive charge and is somewhat polar. This material can be removed by cation exchange or chemical coagulation at high pH with either lime or ferric salts. About 60 percent of the SON is predominantly neutral in charge. One-third of this appears to be polar and difficult to remove by physical processes. The other two-thirds is non-polar and can be removed by activated-carbon adsorption, or partially by coagulation with lime or ferric salts. About 25 percent of the SON is dominated by a negative charge, and except for this, is relatively non-polar. This material can be removed by activated-carbon adsorption or coagulation with alum or ferric salts at a neutral or slightly acidic pH. The remainder of the SON appears to represent larger materials, perhaps colloidal, which are removed by all coagulants and by activated-carbon adsoprtion.

A similar operational analysis of the fractions comprising SCOD was carried out. There was considerable similarity between the distribution of SON and SCOD fractions. The major exception was that a larger fraction of SON than SCOD was represented by the positively charged materials, as might be expected since amino groups tend to carry a positive charge. Separation of soluble organics into fractions in the above way is useful when considering the potential of different treatment processes. Although analysis for specific compounds may be of more value when considering potential health implications of the organic materials, such analysis for a large portion of the SON materials would be difficult with currently available procedures.

CONTROL OF SON

Physical, chemical, and biological methods were evaluated to determine their potential for reducing the concentration of SON in treated effluents. The biological methods involved an evaluation of activated-sludge operational procedures to maximize removal of SON in the original wastewater and to minimize the biological production of SON. It was determined that a detention time and operation representative of conventional design and operation satisfied this goal. With minicipal wastewaters, a detention time of about 6 hours which corresponds to a solids retention time or sludge age of 4 to 10 days would result in minimal effluent SON. SON production is greatest during system start-up and following upsets, and least during good steady-state operation. Long solids retention times also result in more SON production through organism decay. Thus, good treatment plant operation with normal conventional loadings tends to minimize the concentration of effluent SON. Such operation also minimizes the concentration of effluent SCOD.

The physical and chemical methods evaluated included chemical coagulation, activated-carbon adsorption, ion exchange, chlorination, and ozonation. In general it was found that the different processes removed different SON fractions. Thus, a combination of processes was generally capable of removing more SON than a single process alone. These same observations apply to SCOD removal.

As already discussed under SON Characteristics, SON was operationally divided into various fractions which are particularly useful when considering removal by chemical coagulation, activated-carbon adsorption, and ion exchange. These results are summarized in Figure 7 which should be reviewed for reference. Activated-carbon adsorption is capable of removing the non-polar fraction which constitutes about 70 percent of the SON and 80 percent of the SCOD. Cation exchange is particularly useful for selectively removing the positively charged fraction of SON. At a neutral pH it removes about 10 percent of the SON and 5 percent of the SCOD. If wastewater pH is lowered to about 2, nitrogenous materials tend to become further protonated and more positive in charge so that cation exchange can then remove about 40 percent of the SON and 20 percent of the SCOD. The materials removed appear largely to be nucleic acid degradation products and perhaps some amino acids. Cation exchange in general removes a larger fraction of SON than of SCOD. Anion exchange, on the other hand, is not very effective for SON removal, although it is reasonably good for SCOD removal. The resin used appeared to be most effective on a predominantly neutral fraction of organics which was partially polar and partially non-polar. Only about 10 percent of the SON was removed by anion exchange, while about 30 percent of the SCOD was removed. This removal was independent of pH.

Chemical coagulation at neutral or slightly acidic pH was effective in removal of a portion of the soluble organic material which was also removed by activated-carbon adsorption. Both alum and ferric chloride under these conditions removed a larger molecular weight fraction and a negatively charged fraction which comprised about 30 percent of both the SON and SCOD. In addition, ferric chloride removed 15 percent of the non-polar SON. At high pH ferric chloride also removed the positive fraction but not the negative fraction. The fractions removed by lime and ferric chloride under these conditions were about the same.

These results indicate that a combination of activated-carbon adsorption, and either lime coagulation or cation exchange were capable of removing about 85 percent of the SON and SCOD. The non-removed remainder represented a polar fraction of organics which was neutral in charge and not removed by any of these particular processes. In order to obtain the maximum removal noted, large dosages of chemical coagulants or activated carbon would be required. Use of these particular processes specifically to remove SON would be expensive, and should only be attempted if removal is absolutely necessary and justified.

Several strong oxidizing chemicals were evaluated. Large dosages of potassium permanganate and hydrogen peroxide at pH of 10 or higher were capable of removing 20 to 30 percent of the SON, but none of the SCOD. Thus they appear to act simply by deamination to form ammonia and nitrogen-free organics. Large concentrations of free chlorine residual were effective in removing about 40 percent of the SON, but no more than 20 percent of the SCOD. On the other hand, ozonation removed only 14 percent of the SON, but almost 50 percent of the SCOD. Thus, chlorination appears to oxidize the nitrogenous portion of the organics, while ozone is more effective in oxidation or the carbonaceous portion of the organic molecules. Interestingly, preozonation of a sample made it much more susceptible to chlorination for SON removal. Ozonation also tends to make the organics more susceptible to biological degradation.

ECOLOGICAL SIGNIFICANCE OF SON

This study was initiated to determine the nature, effects, and potential methods for control of SON in effluents from municipal treatment plants. One primary concern was proposals to develop effluent standards to limit the total concentration of nitrogen which could be discharged to receiving waters. Questions have been raised about the desirability of including organic nitrogen in a total nitrogen requirement. This study has provided information on the concentrations of SON which can be expected in secondary effluents, the nature of this material, and the effectiveness of various processes for its control. This study did not consider all of the possible ecological ramifications of SON discharge to receiving waters and does not provide sufficient new knowledge about the nature of the materials comprising SON which could be used to evaluate overall ecological effects. The information can be used, however, to determine the implications of effluent standards in process design, operation, and cost.

In general, the organics comprising SON in effluents from conventionally designed and operated activated-sludge plants are only about 50 percent biodegradable. The biodegradable fraction is oxidized very slowly at rates of one to two percent per day. Thus, after 30 days only about two-thirds of the biodegradable material would be oxidized under normal stream conditions. In this time period, about one-third of the SON would be degraded and released as ammonia, which can then be used by algae. Previous studies suggest that little if any of the secondary effluent SON itself is available for algal growth, although the small portion of SON in the form of amino acids could presumably be used for this purpose. A concern which is worth further research is the ecological effect that chlorination of SON materials may have. Amino acids and nucleic acid degradation products, if chlorinated and then reincorporated into living forms could possibly cause undesirable effects. Evaluation of such potential problems, however, was beyond the scope of this study.

The results of this study indicate that SON is not removed to a significant extent by processes used to control or remove the inorganic forms of nitrogen. In general, the adaptation of specific processes for reduction of SON would be expensive. Thus, in the formulation of standards for nitrogen control in wastewater effluents, it is essential that careful consideration be given to the cost of removal and the potential benefits to be gained, before soluble organic forms of nitrogen are included.

REFERENCES

1. U.S. Public Law 92-500; 92nd Congress, S.2770; Oct. 18, 1972.

2. Nitrogenous Compounds in the Environment, E. M. Mrak, Chairman, Hazardous Materials Advisory Committee, United Stated Environmental Protection Agency, Wash., D.C., Report No. EPA-SAB-73-001 (Dec. 1973).

3. Standard Methods for Examination of Water and Wastewater (1971), 13th edition, American Public Health Association, Inc., New York.

4. Horstkotte, G. A., D. G. Niles, D. S. Parker, and D. H. Caldwell, "Full-scale testing of a water reclamation system," Jour. Water Pollution Control Federation, 46(1), 181 (1974).

5. Hunter, J. V., "Origin of organics from artificial contamination," in Organic Compounds in Aquatic Environments, S. D. Faust and J. V. Hunter, eds., Marcel Dekker, Inc., New York, 1971.

6. Hanson, A. M., and G. F. Lee, "Forms of organic nitrogen in domestic wastewater," Jour. Water Pollution Control Federation, 43(11), 2271 (1971).

7. Fogg, G. E., "Production of extracellular nitrogenous substances by a blue green algae," Proc. Royal Society (London), B139, 372 (1952).

8. Fogg, G. E., "The extracellular products of algae," Oceanographical and Marine Biology Annual Review, 4, 195 (1966).

9. Neilands, J. B., "Hydroxamic acids in nature," Science, 156 1443 (1967).

10. Hunter, S. H., "Inorganic nutrition," Annual Reviews of Microbiology, 26, 313 (1972).

11. Keeney, D. R., J. G. Konrad, and G. Chesters, "Nitrogen distribution in some Wisconsin lake sediments," Jour. Water Pollution Control Federation, 42(3), 411 (1970).

12. Dean, R. B., "Colloids complicate treatment processes," Environmental Science and Technology, 3(9), 820 (1969).

13. Helfgott, T., J. V. Hunter, and D. Rickert, "Analytic and process classification of effluents," Jour. Sanitary Engineering Div., ASCE, 96, SA3, 779 (June 1970).

14. Painter, H. A., M. Viney, and A. Bywaters, "Composition of sewage and sewage effluents," Jour. and Proc. Institute of Sewage Purification (Great Britain), Part 4, 302 (1961).

15. Hunter, J. V., and H. Heukelekian, "The composition of domestic sewage fractions," Jour. Water Pollution Control Federation, 37(8), 1142 (1965).

16. Rickert, D. A., and J. V. Hunter, "General nature of soluble and particulate organics in sewage and secondary effluent," Water Research, 5, 421 (1971).

17. Sastry, C. A., P. V. R. Subrahmanyam, and S. C. Pillai, "Amino acids in treated sewage in India," Sewage and Industrial Wastes, 30(10, 1241 (1958).

18. Kahn, L., and C. Wayman, "Amino acids in raw sewage and sewage effluents," Jour. Water Pollution Control Federation, 36(11), 1368 (1964).

19. Beckman, W. J., R. J. Avendt, T. J. Mulligan, and G. J. Kehrberger, "Combined carbon oxidation-nitrification," Jour. Water Pollution Control Federation, 44(10), 1916 (1972).

20. Adrian, G. W., and W. W. Hodges, Jr., "Pilot scale activated carbon advanced treatment and plans for future recycling at Los Angeles," Proc. 15th Water Quality Conference, Univ. of Illinois, Urbana, Ill., (1973).

21. Esmond, S. E., and H. W. Wolf, "The status of organics--the Dallas Water Reclamation Pilot Plant," Proc. 15th Water Quality Conference, Univ. of Illinois, Urbana, Ill., (1973).

22. Rebhun, M., and J. Manka, "Classification of organics in secondary effluents," Environmental Science and Technology, 5, 606 (1971).

23. Bunch, R. L., E. F. Barth, and M. B. Ettinger, "Organic materials in secondary effluents," Jour. Water Pollution Control Federation, 33(2), 122(1961).

24. Wastewater Engineering, Metcalf and Eddy, Inc., McGraw-Hill, Inc., New York (1972).

25. Rudolph, W., and N. S. Chamberlin, "Indole and skatole in sewage," Industrial and Engineering Chemistry, 24, 111 (1932).

26. Manka, J., M. Rebhun, A. Mandelbaum, and A. Bertinger, "Characterization of organics in secondary effluents," Environmental Science and Technology, 8, 1017 (1974).

27. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," Jour. Biological Chemistry, 193, 265 (1951).

28. DeWalle, F. B., and E. S. K. Chian, "Removal of organic matter by activated carbon columns," Jour. Environmental Engineering Div., ASCE, 100, EE5, 1089 (Oct. 1974).

29. Gunsalus, I. C., and C. W. Shuster, "Energy-yielding metabolism in bacteria," in The Bacteria. Volume II: Metabolism, I. C. Gunsalus and R. Y. Stanier, eds., 1-58, Academic Press, New York, 1961.

30. Gale, E. F., "The synthesis of proteins and nucleic acids," in The Bacteria. Volume III: Biosynthesis, I. C. Gunsalus and R. Y. Stanier, eds., 471-576, Academic Press, New York, 1961.

31. Magasanik, B., "Biosynthesis of purine and pyrimidine nucleotides," in The Bacteria. Volume III: Biosynthesis, I. C. Gunsalus and R. Y. Stanier, eds., 295-334, Academic Press, New York, 1961.

32. Herbert, H., "The chemical composition of micro-organisms as a function of their environment," Symposium for Society of General Microbiology, 11, 391-416 (1961).

33. Siegel, A., and E. T. Degens, "Concentration of dissolved amino acids from saline waters by ligand-exchange chromatography," Science, 151, 1098 (March 1966).

34. Minear, R. A., "Characterization of naturally occurring dissolved organophosphorus compounds," Environmental Science and Technology, 6 (5), 431 (1972).

35. Bremner, J. M., "Studies on soil organic matter. I. The chemical nature of soil organic nitrogen," Jour. Agricultural Science, 39, 183 (1949).

36. Bremner, J. M., "Studies on soil humic acids. I. The chemical nature of humic nitrogen," Jour. Agricultural Science, 46, 247 (1955).

37. Stevenson, F. J., and K. M. Goh, "Infrared spectra of humic acids and related substances," Geochimica et Cosmochimica Acta, 35, 471 (1971).

38. Otsuki, A., and T. Hanya, "Some precursors of humic acid in recent lake sediment suggested by infrared spectra," Geochimica et Cosmochimica Acta, 31, 1505 (1967).

39. Ishiwatari, R., "Structural characteristics of humic substances in recent lake sediments," in Advances in Organic Geochemistry, G. D. Hobson and G. C. Speers, eds., Pergammon Press, Inc., New York, 1970.

40. Hodge, J. E., "Chemistry of browning reactions in model systems," Jour. Agricultural and Food Chemistry, 1, 928 (1953).

41. Stevenson, F. J., and S. N. Tilo, "Nitrogenous constituents of deep-sea sediments," in Advances in Organic Geochemistry, G. D. Hobson and G. C. Speers, eds., Pergammon Press, Inc., New York, 1970.

42. Dugan, P. R., "Bioflocculation and the accumulation of chemicals by floc-forming organisms," U.S.E.P.A. Report No. 600/2-75-032 (1975).

43. Baier, R. E., E. G. Shafrin, and W. A. Zisman, "Adhesion: mechanisms that assist or impede it," Science, 162, 1360 (1968).

44. Putnam, F. W., and H. Neurath, "Complex formation between synthetic detergents and proteins," Jour. Biological Chemistry, 150, 263 (1943).

45. Pitter, P., "Determination of biological degradability of organic substances," Water Research, 10, 231-235 (1976).

46. Lowry, O. H., N. J. Rosebraugh, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," Jour. Biological Chemistry, 193, 256 (1951).

47. Pfeffer, J. T., "Reclamation of energy from organic refuse," Progress report on work at Univ. of Illinois, Dept. of Civil Engineering on EPA-R-800776, April 1973.

48. Methods for Chemical Analysis of Water and Wastewater, U.S.E.P.A. Report No. 16020-07/71 (1971).

49. Howe, L. H., and C. W. Holley, "Comparisons of mercury(II) chloride and sulfuric acid as preservatives for nitrogen forms in water samples," Environmental Science and Technology, 3, 478 (1969).

50. Eckhoff, D. W., and D. Jenkins, "Transient loading effects in the activated sludge process," Proceedings of the 3rd International Conference of Water Pollution Research, Munich, 1966.

51. McCarty, P. L., R. Schertenleib, and S. Niku, "Preproject water quality evaluation for the Palo Alto Water Reclamation Facility," Technical Report No. 206, Dept. of Civil Engineering, Stanford Univ., April, 1976.

52. Folin, O., and V. Ciocalteu, "On tyrosine and tryptophane determination in proteins," Jour. Biological Chemistry, 73, 627 (1927).

53. Stumm, W., and C. R. O'Melia, "Stoichiometry of coagulation," Jour. American Water Works Association, 60(5), 514 (1968).

54. Black, A. P., and S. A. Hannah, "Electrophoretic studies of turbidity removal by coagulation with aluminum sulfate," Jour. American Water Works Association, 53(4), 438 (1961).

55. Alexander, M., "Biodegradation: problems of molecular recalcitrance and microbial fallibility," Advances in Applied Microbiology, 7, 35-80 (1965).

56. Alexander, M., "Nonbiodegradable and other recalcitrant molecules," Biotechnology and Bioengineering, 15, 611-647 (July 1973).

57. Alexander, M., "Environmental and microbiological problems arising from recalcitrant molecules," *Microbial Ecology*, *2*, 17-27 (1975).

58. Marion, C. V., and G. W. Malaney, "Ability of activated sludge microorganisms to oxidize aromatic compounds," *Proc. 18th Ind. Waste Conference*, Purdue Univ., Lafayette, Indiana, 1963.

59. Symons, J. M., R. E. McKinney, R. M. Smith, and E. J. Donovan, Jr., "Degradation of nitrogen-containing compounds by activated sludge," in *Advances in Biological Water Treatment*, W. W. Eckenfelder and Brother J. McCabe, eds., Pergamon Press, New York, 1963.

60. Dugan, P. R., *Biochemical Ecology of Water Pollution*, Plenum Press, New York, 1972.

61. Rogers, H. J., "The dissimilation of high molecular weight substances," in *The Bacteria. Volume II: Metabolism*, I. C. Gunsalus and R. Y. Stanier, eds., 257-318, Academic Press, New York, 1961.

62. Chudoba, J., J. Cervenka, and M. Zima, "Residual organic matter in activated sludge process effluents. IV. Treatment of municipal sewage," Scientific Papers of the Institute of Chemical Technology (Czech.), *Technology of Water*, *F15*, 5-19 (1969).

63. Saunders, F. M., "Effect of cell residence time on the extracellular organic compounds in activated sludge suspensions," Ph.D. Thesis, Dept. of Civil Engineering, Univ. of Illinois, Urbana, Illinois, 1975.

64. Grady, C. P. L., Jr., L. J. Harlow, and R. R. Riesing, "Effect of growth rate and influent substrate concentration on effluent quality from chemostats containing bacteria in pure and mixed culture," *Biotechnology and Bioengineering*, *14*, 391-410 (1972).

65. Bisogni, J. J., Jr., and A. W. Lawrence, "Relationships between biological solids retention time and settling characteristics of activated sludge," *Water Research*, *5*, 753-763 (1971).

66. Sherrard, J. H., and E. D. Schroeder, "Relationship between the observed cell yield coefficient and mean cell residence time in the completely mixed activated sludge process," *Water Research*, *6*, 1039-1049 (1972).

67. Grady, C. P. L., Jr., A. F. Gaudy, Jr., and E. T. Gaudy, "Control mechanisms operative in a natural microbial population selected for its ability to degrade ℓ-Lysine. I. Effect of glucose in batch systems," *Applied Microbiology*, *18*, 776-784 (Nov. 1969).

68. Grady, C. P. L., Jr., and A. F. Gaudy, Jr., "Control mechanisms operative in a natural microbial population selected for its ability to degrade ℓ-lycine. II. Effects of fructose and ribose in batch systems," *Applied Microbiology*, *18*, 785-789 (Nov. 1969).

69. Grady, C. P. L., Jr., and Gaudy, A. F., Jr., "Control mechanisms operative in a natural microbial population selected for its ability to degrade ℓ-lycine. III. Effects of carbohydrates in continuous-flow systems under shock load conditions," Applied Microbiology, 18, 790-797 (Nov. 1969).

70. LaMer, V. K., and T. W. Healy, "Adsorption-flocculation reactions of macromolecules at the solid-liquid interface," Reviews of Pure and Applied Chemistry, 13, 112 (1963).

71. Ries, H. E., and B. L. Mayers, "Flocculation mechanism: charge neutralization and bridging," Science, 160, 1449 (1968).

72. Black, A. P., "Basic mechanisms of coagulation," Jour. American Water Works Association, 52(4), 492 (1960).

73. Parkin, G. F., and P. L. McCarty, "The nature, ecological significance, and removal of soluble organic nitrogen in treated agricultural wastewaters," Technical report No. 180, Stanford Univ., Dept. of Civil Engineering (Sept. 1973).

74. Malhotra, S. K., G. F. Lee, G. A. Rohlich, "Nutrient removal from secondary effluent by alum flocculation and lime precipitation," International Journal of Air and Water Pollution, 8, 487 (1964).

75. Wolf, H., "Water-borne nitrogenous materials from municipal areas," in Nitrogenous Compounds in the Environment, E. M. Mrak, Chairman, Hazardous Materials Advisory Committee, U.S.E.P.A., Washington, D.C., Report No. EPA-SAB-73-001 (Dec. 1973).

76. Rebhun, M., N. Narkis, and A. M. Wachs, "Effect of polyelectrolytes in conjunction with bentonitic clay on contaminants removal from secondary effluents," Water Research, 3, 345 (1969).

77. Yopps, J. A., and D. W. Fuerstenau, "The zero point of charge of alpha-alumina," Jour. Colloid Science, 19, 61 (1964).

78. Healy, T. W. "Selective adsorption of organics on inorganic surfaces," in Organic Compounds in Aquatic Environments, S. D. Faust and J. V. Hunter, eds., Marcel Dekker, Inc., New York, 1971.

79. Parks, G. A., "The isoelectric points of solid oxides, solid hydroxides, and aqueous hydroxo complex systems," Chemical Reviews, 65, 177 (1965).

80. Parks, G. A., "Aqueous surface chemistry of oxides and complex oxide minerals," in Equilibrium Concepts in Natural Water Systems, Advances in Chemistry Series, No. 67, American Chemical Society, Washington, D.C., 1967.

81. Black, A. P., and R. F. Christman, "Electrophoretic studies of sludge Particles produced in lime-soda softening," Jour. American Water Works Association, 53, 737 (1961).

82. Faust, S. D., and M. C. Manger, "Distribution of electromobility values of particulate matter in domestic waste water," *Air and Water Pollution*, *9*, 565 (1965).

83. Sawyer, C. N., and P. L. McCarty, *Chemistry for Sanitary Engineering*, 2nd edition, McGraw-Hill, Inc., New York, 1967.

84. Busch, P. L., and W. Stumm, "Chemical interactions in the aggregation of bacteria--bioflocculation in waste treatment," *Environmental Science and Technology*, *2*, 49 (1968).

85. *Process Design Manual for Phosphorus Removal*, U.S.E.P.A., Report No. EPA-625/1-76-001a, April 1976.

86. Green, O., D. VanDan, B. LaBeau, T. L. Campbell, and S. L. Daniels, "Ferris chloride and organic polyelectrolytes for the removal of phosphorus," EPA Report No. 670/2-73-103 (1974).

87. Boggia, C., and G. L. Herriman, "Pilot plant operation at Warren, Michigan," *Proc. of the 43rd Annual Conference of the Michigan Pollution Control Association*, 1968.

88. Lengweiller, H., W. Buser, and W. Feitknecht, "The determination of the solubility of iron(III) hydroxides with FE^{59}," *Helvitica Chimica Acta*, *44*, 796 (1961).

89. Stumm, W., and J. J. Morgan, "Chemical aspects of coagulation," *Jour. American Water Works Association*, *54*, 971 (1962). Discussion, p. 992, A. P. Black.

90. Collins, T. T., H. L. Davis, and B. W. Rowland, "A study of the behavior of alumina in relation to sizing," *Paper Trade Journal*, *113*, 94 (1941).

91. Cleaver, C. S., and H. G. Cassidy, "Chromatographic adsorption of amino acids on organic exchange resins," *Jour. American Chemical Society*, *72*, 1147 (1950).

92. Rebhun, M., and W. J. Kaufman, "Removal of organic contaminants. Sorption of organics by synthetic resins and activated carbon," SERL Report No. 67-9, U.S. Berkeley, Calif., Dec. 1967.

93. Horvath, R. S., "Microbial co-metabolism and the degradation of organic compounds in nature," *Bacteriological Reviews*, *36*, 146-155 (June 1972).

94. McCarty, P. L., "Energetics of organic matter degradation," in *Water Pollution Microbiology*, R. Mitchell, ed., Wiley-Interscience, New York, 1972.

95. Nissenbaum, A., and I. R. Kaplan, "Chemical and isotropic evidence for the in-situ origin of marine humic substances," *Limnology and Oceanography*, *17*, 570-582 (July 1972).

96. Nissenbaum, A., "The organic geochemistry of marine and terrestrial humic substances: Implications of carbon and hydrogen isotope studies," in Advances in Organic Geochemistry 1973, B. Tissot and F. Bienner, eds., Editions Technip, Paris, 1974.

97. Welte, D., "Recent advances in organic geochemistry of humic substances and kerogen. A review," in Advances in Organic Geochemistry 1973, B. Tissot and F. Bienner, eds., Editions Technip, Paris, 1974.

98. Vogels, G. D., and C. van der Drift, "Degradation of purines and pyrimidines by microorganisms," Bacteriological Reviews, 40, 403-468 (June 1976).

99. Dawes, E. A., and D. W. Ribbons, "Some aspects of the endogenous metabolism of bacteria," Bacteriological Reviews, 28, 126-149 (June 1964).

100. Gronlund, A. F., and J. J. R. Campbell, "Nitrogenous substrates of endogenous respiration in Pseudomonas aeruginosa," Journal of Bacteriology, 86, 58-66 (July 1963).

101. McGregor, W. C., and R. K. Finn, "Factors affecting the flocculation of bacteria by chemical additives," Biotechnology and Bioengineering, 11, 127-138 (March 1969).

102. Moore, S., D. H. Spackman, and W. H. Stein, "Chromatography of amino acids on sulfonated polystyrene resins," Analytical Chemistry, 30(7), 1185 (1958).

103. Moore, S., and W. H. Stein, "Chromatography of amino acids on sulfonated polystyrene resins," Jour. Biological Chemistry, 192 663 (1951).

104. Hamilton, P. B., "Ion exchange chromatography of amino acids," Analytical Chemistry, 30(5), 914 (1958).

105. Buchanan, D. L., "Desalting amino acid solutions by displacement with piperidine," Analytical Chemistry, 29, 1877 (1957).

106. Cleaver, C. S., and H. G. Cassidy, "Chromatographic adsorption of amino acids on organic exchange resins," Jour. American Chemical Society, 72, 1147 (1950).

107. Brown, D. M., "The separation of nucleic acid degradation products," in Ion Exchange and Its Applications, Society of Chemical Industry, London, 1955.

108. Cohn, W. E., "The anion-exchange separation of ribonucleotides," Jour. American Chemical Society, 72, 1471 (1950).

109. Thomas, T. O., and R. D. Batt, "Degradation of cell constituents by starved Streptococcus lactis in relation to survival," Journal General Microbiology, 58, 347-362 (Nov. 1969).

110. Giusti, D. M., R. A. Conway, and C. T. Larson, "Activated carbon adsorption of petrochemicals," Jour. Water Pollution Control Federation, 46(5), 947 (1974).

111. Culp, R. L., and G. L. Culp, Advanced Wastewater Treatment, Van Nostrand Reinhold, New York (1971).

112. Strange, R. E., H. E. Wade, and A. G. Ness, "The catabolism of proteins and nucleic acids in starved Aerobacter aerogenes," Biochemical Journal, 86, 197-203 (1963).

113. Saier, M. H., Jr., B. U. Feucht, M. T. McCaman, "Regulation of intro-cellular adenosine cyclic 3':5'-monophosphate levels in Escherichia coli and Salmonella typhimurium," Journal of Biological Chemistry, 250, 7593-7661 (Oct. 10, 1975).

114. Neijssel, O. M., and D. W. Tempest, "The role of energy-spilling reactions in the growth of Klebsiella aerogenes NCTC 418 in aerobic chemostat culture," Ach. Microbiol., 110, 305-311 (1976).

115. Thompson, J., "Characteristics and energy requirements of an α-aminoisobutyric acid transport system in Streptococcus lactis," Journal of Bacteriology, 127, 719-730 (Aug. 1976).

116. Postgate, J. R., and J. R. Hunter, "Accelerated death of Aerobacter aerogenes starved in the presence of growth-limiting substrates," Journal of General Microbiology, 34, 459-473 (May 1964).

117. Calcott, P.H., and J. R. Postgate, "On substrate-accelerated death in Klebsiella aerogenes," Journal of General Microbiology, 70, 115-122 (March 1972).

118. Pirt, S. John, Principles of Microbe and Cell Cultivation, John Wiley and Sons, New York, 1975.

119. Garten, V. A., and D. E. Weiss, "The ion- and electron-exchange properties of activated carbon in relation to its behavior as a catalyst and adsorbent," Reviews of Pure and Applied Chemistry, 7, 69 (1957).

120. Snoeyink, V. L., and W. J. Weber, Jr., "The surface chemistry of active carbon. A discussion of structure and surface functional groups," Environmental Science and Technology, 1, 228 (1967).

121. Coughlin, R. W., and F. S. Ezra, "Role of surface acidity in adsorption of organic pollutants on the surface of carbon," Environmental Science and Technology, 2, 291 (1968).

122. Weber, W. J., Jr., and J. C. Morris, "Kinetics of adsorption on carbon from solution," Jour. Sanitary Engineering Div., ASCE, 89, SA2, 31 (1963).

123. Weber, W. J., Jr., and J. C. Morris, "Equilibria and capacities for adsorption on carbon," Jour. Sanitary Engineering Div., ASCE, 90, SA3, 79 (1964).

124. Urano, K., and K. Nakamura, "The behavior of heavy metals in the regeneration process of sewage treatment activated carbon," Gesuido Kyokai-Shi, 115(10), 19 (1973), Japan.

125. Spicher, R. G., and R. T. Skrinde, "Potassium permanganate oxidation of organic contaminants in water supplies," Jour. American Water Works Association, 55(11), 1174 (1963).

126. Slechta, A. F., and G. L. Culp, "Water reclamation studies at the South Tahoe PUD," Jour. Water Pollution Control Federation, 39, 787 (1967).

127. Parkhurst, J. D., et al., "Pomona activated carbon pilot plant," Jour. Water Pollution Control Federation, 39, R70 (1967).

128. Kim, B. R., V. L. Snoeyink, and F. M. Saunders, "Influence of activated sludge CRT on adsorption," Jour. Environmental Engineering Div., ASCE, EE1, 55 (1976).

129. Keller, J. V., "Activated carbon adsorption of soluble organic nitrogen compounds in municipal secondary effluent," Engineer Thesis, Stanford University, Feb. 1976.

130. DeWalle, F. B., and E. S. K. Chian, "The kinetics of formation of humic substances in activated sludge systems and their effect on flocculation," Biotechnology and Bioengineering, 16(6), 739 (1974).

131. Williams, D. B., "The organic nitrogen problem," Jour. American Water Works Association, 43, 837 (1951).

132. Wright, N. C., "The action of hypochlorite on amino acids and proteins," Biochemistry Journal, 20, 524 (1926).

133. Wright, N. C., "The action of hypochlorite on amino acids and proteins. The effect of acidity and alkalinity," Biochemistry Journal, 30, 1661 (1936).

134. Lawrence, A. W., W. S. Howard, and K. A. Rubin, "Ammonia nitrogen removal from wastewater effluents by chlorination," Presented at 48th Mid-Atlantic Industrial Waste Conference, Univ. of Delaware, Nov. 19, 1970.

135. Pressley, T. A., D. F. Bishop, and S. G. Roan, "Nitrogen removal by breakpoint chlorination," Presented at 160th national meeting of the American Chemical Society, Chicago, Ill., Sept., 1970.

136. Murphy, K. L., R. Zaloum, and D. Fulford, "Effect of chlorination practice on soluble organics," Water Research, 9, 389 (1975).

137. Sung, R. D., "Effects of organic constituents in wastewater on the chlorination precess," Ph.D. Thesis, Univ. of California, Davis, 1975.

138. Taras, M. J., "Preliminary studies on the chlorine demand of specific compounds," Jour. American Water Works Association, 42(5), 462 (1950).

139. Taras, M. J., "Effect of free residual chlorination on nitrogen compounds in water," Jour. American Water Works Association, 45(1), 47 (1953).

140. Chudoba, J., and J. Dalesicky, "Chemical oxygen demand of some nitrogenous heterocyclic compounds," Water Research, 7, (1973).

141. Morris, J. C., "Formation of halogenated organics by chlorination of water supplies (a review), "National Technical Information Service Report No. PB-241 511 (March 1975).

142. Hewes, C. G., and R. R. Davison, "Kinetics of ozone decomposition and reaction with organics in water," Jour. American Institute of Chemical Engineering, 17(1), 141 (1971).

143. Peleg, M., "Chemistry of ozone in treatment of water," Water Research, 10(5), 361 (1976).

144. Roan, S. G., D. F. Bishop, and T. A. Pressley, "Laboratory ozonation of municipal wastewaters," EPA Report No. 670/2-73-075 (1973).

145. Nebel, C., et al., "Ozone disinfection of industrial-municipal secondary effluents," Jour. Water Pollution Control Federation, 45, 2493 (1973).

146. Kirk, B. S., R. McNabney, and C. S. Wynn, "Pilot plant studies of tertiary treatment with ozone," in Ozone in Water and Wastewater Treatment, F. L. Evans, III, ed., Ann Arbor Science Publishers, Inc. Ann Arbor, Michigan (1972).

147. Singer, P. C., and W. B. Zilli, "Ozonation of ammonia in wastewater," Water Research, 9, 127 (1975).

148. Demain, A. L., R. W. Burg, and D. Hendlin, "Excretion and degradation of ribonucleic acids by Bacillus subtilis," Journal of Bacteriology, 89 (March 1965).

149. Nichikowa, S., and M. Kurivama, "Nucleic acid as a component of mucilage in activated sludge," Water Research, 2, 811 (1968)

150. Harold, F. M., "Conservation and transformation of energy by bacterial membranes," Bacteriological Reviews, 36, 172 (1972).

151. Payne, J. W., "Peptides and micro-organisms," Advances in Microbial Physiology, 13, 56-113 (1976).

152. Christensen, H. N., Biological Transport, W. A. Benjamin, Inc., Reading, Mass., 1975.

153. Boylen, C. W., and J. C. Ensign, "Intracellular substrates for endogenous metabolism during long-term starvation of rod and spherical cells of Arthrobacter crystallopoietes," Journal of Bacteriology, 103, 578-587 (Sept. 1970).

154. Bailey, P. S., "The reactions of ozone with organic compounds," Chemical Reviews, 58, 925 (1958).

155. Bailey, P. S., "Organic groupings reactive toward ozone-mechanisms in aqueous media," in Ozone in Water and Wastewater Treatment, F. L. Evans, III, ed., Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, 1972.

156. Bailey, P. S., "Reactivity of ozone with various organic functional groups important to water purification," in First International Symposium on Ozone for Water and Wastewater Treatment, R. G. Rice and M. E. Browning, eds., International Ozone Institute, Waterbury, Conn., 1975.

157. Burleigh, I. G., and E. A. Dawes, "Studies on the endogenous metabolism and senescence of starved Sarcina lutea," Biochemical Journal, 102, 236-250 (1967).

158. Maggiolo, A., and S. J. Niegowski, "Preparation of tertiary amine oxides by ozonation," Advances in Chemistry Series, 21, 202 (1959).

159. Razumovskii, S. D., A. L. Buchachenko, A. B. Shapiro, E. G. Rozantsev, and G. E. Zaikov, "The formation of nitroxyl radicals in the reaction of amines with ozone," Doklady Chemistry, 183, 1086 (1968).

160. Hewes, C. G., and R. R. Davison, "Kinetics of ozone decomposition and reaction with organics in water," Jour. American Institute of Chemical Engineering, 17(1), 141 (1971).

161. Bailey, P. S., J. E. Keller, D. A. Mitchard, and H. M. White, "Ozonation of amines," Advances in Chemistry Series, 77, 58 (1968).

162. Bailey, P. S., D. A. Mitchard, and A. Y. Khashab, "Ozonation of amines. II. The competition between amine oxide formation and side-chain oxidation," Jour. Organic Chemistry, 33(7), 2675 (1968).

163. Bailey, P. S., and J. E. Keller, "Ozonation of amines. III. t-Butylamine," Jour. Organic Chemistry, 33(7), 2680 (1968).

164. Bailey, P. S., J. E. Keller, and T. P. Carter, Jr., "Ozonation of amines. IV. Di-ti-bytylamine," Jour. Organic Chemistry, 35, 2777 (1970).

165. Bailey, P. S., and J. E. Keller, "Ozonation of amines. V. Di-t-butyl nitroxide," Jour. Organic Chemistry, 35, 2782 (1970).

166. Erickson, R. E., P. J. Andrulis, Jr., J. C. Collins, M. L. Lungle, and G. D. Mercer, "Mechanism of ozonation reactions. IV. Carbon-nitrogen double bonds," Jour. Organic Chemistry, 34, 2961 (1960).

167. Wibaut, J. P., "Ozonolysis of pyrroles, furans, and γ-pyrones in connection with reactivity of bonds in ring system," Advances in Chemistry Series, 21, 153 (1959).

168. Mudd, J. B., R. Leavitt, A. Ongren, and T. T. McManus, "Reaction of ozone with amino acids and proteins," Atmospheric Environment, 3, 669 (1969).

169. Delman, A. D., A. E. Ruff, B. B. Simms, and A. R. Allison, "Influence of Molecular structure and substituents on antiozonant properties of aryl diamine compounds," Advances in Chemistry Series, 21, 176 (1959).

170. Mosher, W. A., "Structural relationships in addition of ozone to double bonds," Advances in Chemistry Series, 21, 140 (1959).

171. Medalia, A. I., "Test for traces of organic matter in water," Analytical Chemistry, 23(9), 1318 (1951).

172. Felbeck, G. T., Jr., "Structural chemistry of soil humic substances," Advances in Agronomy, 17, 327 (1965).

173. Spicher, R. G., and R. T. Skrinde, "Effects of potassium permanganate on pure organic compounds," Jour. American Water Works Association, 57(4), 472 (1965).

174. Denisov, E. T., V. M. Solyanikov, and A. L. Alexandrov, "Ionic catalysis in chain oxidation of alcohols," Advances in Chemistry Series, 75, 112 (1968).

175. Schertenleib, R., "Laboratory investigations for the Palo Alto Water Reclamation Plant," Engineer's Thesis, Stanford University, 1977.

176. Williams, R. T., in Biological Oxidation of Nitrogen in Organic Molecules, J. W. Bridges, J. W. Gorrod, and D. V. Parke, eds., Halsted Press, New York, 1972.

177. Postgate, J. R., and J. R. Hunter, "The survival of starved bacteria," Journal of General Microbiology, 29, 233 (1962).

178. Radar, R. L., and J. Hochstadt, "Regulation of purine utilization in bacteria. VII. Involvement of membrane-associated nucleoside phosphorylases in the uptake and the base-mediated loss of the ribose moiety of nucleosides by Salmonella typhimurium membrane vesicles," Journal of Bacteriology, 128, 290 (1976).

179. Sontheimer, H., "New experiences with ozone in Germany," in *First International Symposium on Ozone for Water and Wastewater Treatment*, R. G. Rice and M. E. Browning, eds., International Ozone Institute, Waterbury, Conn., 1975.

180. Stern, A. M., and Gasner, L. L., "Degradation of lignin by combined chemical and biological treatment," *Biotechnology and Bioengineering*, *16*, 789 (1974).

181. *CRC Handbook of Biochemistry, Selected Data for Molecular Biology*, H. A. Sober, ed., CRC Press, Cleveland, Ohio, 1970.

182. Lehninger, A. L., *Biochemistry*, Worth Publishers, Inc., New York, 1970.

183. Semmens, M., and J. Gregory, "Selectivity of strongly basic anion exchange resins for organic ions," *Environmental Science and Technology*, *8*(9), 834 (1974).

184. Pavoni, J. L., M. W. Tenney, and W. F. Echelberger, Jr., "Bacterial exocellular polymers and biological flocculation," *Jour. Water Pollution Control Federation*, *44*(3), 414 (1972).

185. Moroney, M. J., *Facts from Figures*, Penguin Books, Inc., Baltimore, Md., 1951.

186. Laitinen, H. A., and W. E. Harris, *Chemical Analysis*, 2nd ed., McGraw-Hill, Inc., New York, 1975.

187. Analytical Quality Control, U.S.E.P.A., Technology Transfer, June 1972.

188. Edwards, Allen L., Statistical Analysis, 3rd ed., Holt, Rinehart, and Winston, Inc., New [illegible] (1969).

189. Strange, R. E., an [illegible]. Dark, "'Substrate-accelerated death' of *Aerobacter aerogenes*, Journal of General Microbiology, 39, 215 (1965).

190. Hunter, S. H., "Inorganic nutrition," Annual Reviews of Microbiology, *26*, 313-346 (1972).

191. Kinoshita, S., *et al.*, "Inorganic nutrition," *Annual Reviews of Microbiology*, *26*, 313 (1972).

192. Smeaton, J. R., and W. H. Elliot, "Selective release of ribonuclease-inhibitor from *B. subtilis* cells by cold shock treatment," *Biochemical and Biophysical Research Communications*, *26*, 75 (1967).

193. Rogers, D., "Osmotic pools in *Escherichia celi*," *Science*, *159*, 531 (1968).

194. Heppel, L., "Selective release of enzymes from bacteria," *Science*, *156*, 1451 (1967).

195. Nossal, N. G., and L. A. Heppel, "The release of enzymes by osmotic shock from E. coli in exponential phase," Journal of Biological Chemistry, 241, 3055 (1966).

196. Lampen, J. O., "Secretion of enzymes by microorganisms," in Function and Structure of Microorganisms, Proc. 15th Symposium of Society for General Microbiology, London, 1965.

197. Clark, V. L., D. E. Peterson, and R. W. Bernlohr, "Changes in free amino acid production and intracellular amino acid pools of Bacillus licheniformis as a function of culture age and growth media," Journal of Bacteriology, 112, 715-725 (1972).

198. Kjeldaard, N. O., "Regulation of nucleic acid and protein formation in bacteria," Advances in Microbial Physiology, 1, 39-95 (1967).

199. Strange, R. E., F. A. Dark, and A. G. Ness, "The survival of stationary phase Aerobacter aerogenes stored in aqueous suspension," Journal of General Microbiology, 25, 61 (1961).

200. Lawrence, A. W., and P. L. McCarty, "Unified basis for biological treatment design and operation," Jour. Sanitary Engineering Div., ASCE, 96, 757-778 (June 1970).

201. Wuhrman, K., "Microbial aspects of water pollution control," Advances in Applied Microbiology, 6, 119-151 (1964).

202. Randtke, S. J.,"Occurrence and removal characteristics of soluble organic nitrogen in municipal secondary effluent," Ph.D. Thesis, Stanford Univ., Stanford, California, 1977.

203. Gaudy, A. F., Jr., P. Y. Yang, and A. W. Obayashi, "Studies on the total oxidation of activated sludge with and without hydrolytic pretreatment," Jour. Water Pollution Control Federation, 43, 40 (1971).

204. Gaudy, A. F., Jr., M. Ramanathan, P. Y. Yang, and T. V. DeGeare, "Studies on the operational stability of the extended aeration process," Jour. Water Pollution Control Federation, 42, 165 (1970).

205. Monod, J., "The growth of bacterial cultures," Annual Reviews of Microbiology, 3, 371-394 (1949).

206. Strominger, J. L., "Biosynthesis of bacterial cell walls," in The Bacteria. Volume III. Biosynthesis, I. C. Gunsalus and R. Y. Stanier, eds., 413-470, Academic Press, New York, 1961.

207. Pike, E. B., and C. R. Curds, "The microbial ecology of the activated sludge process," in Microbial Aspects of Pollution, G. Sykes and F. A. Skinner, eds., Academic Press, New York, 1971.

208. Jewell, W. J., and P. L. McCarty, "Aerobic decomposition of algae," Environmental Science and Technology, 5, 1023 (Oct. 1971).

209. Bender, M. E., W. R. Matson, and R. A. Jordan, "On the significance of metal complexing agents in secondary sewage effluents," *Environmental Science and Technology*, *4*, 520 (1970).

210. "Characterization and separation of secondary effluent components by molecular weight," 16020 FEN 03/71, *Water Pollution Control Research Series*, Arthur D. Little, Inc., Environmental Protection Agency, Washington, D.C. (1971).

211. Zuckerman, M. M., and A. H. Molof, "High quality reuse by chemical-physical wastewater treatment," *Jour. Water Pollution Control Federation*, *42*, 437 (1970).

212. Irvine, R. L., and D. J. Schaezler, "Kinetic analysis of data from biological systems," *Jour. Sanitary Engineering Div.*, *ASCE*, *97*, 409 (1971).

213. Murphy, K. L., M. J. Sedivy, and G. A. Vigers, "Residual organics following biological treatment," *Proc., 6th Canadian Symposium on Water Pollution Research*, Univ. of Toronto, Toronto, Canada (1971).

214. Chudoba, J., "Residual organic matter in activated sludge process effluents. I. Degradation of saccharides, fatty acids, and amino acids under batch conditions," Scientific Papers of the Institute of Chemical Technology (Czech.), *Technology of Water*, *F12*, 39 (1967).

215. Gaudy, A. F., Jr., and R. S. Englebrecht, "Basic biochemical considerations during metabolism in growing vs. respiring systems," in *Advances in Biological Waste Treatment*, W. W. Eckenfelder and Brother J. McCabe, eds., Pergamon Press, New York, 1963.

216. Komolrit, K., K. C. Goel, and A. F. Gaudy, Jr., "Regulation of exogeneous nitrogen supply and its possible applications to the activated sludge process," *Jour. Water Pollution Control Federation*, *39*, 251 (1967).

217. Rao, B. S., and A. F. Gaudy, Jr., "Effect of sludge concentration on various aspects of biological activity in activated sludge," *Jour.* Water Pollution Control Federation, 38, 794 (1966).

218. Spiegel, M. R., Schaums's Outline of Theory and Problems of Statistics, McGraw-Hill Book Co., New York, 1961.

219. *Sephadex: Gel Filtration in Theory and Practice*, Pharmacia Fine Chemicals, Inc., Piscataway, New Jersey, 1975.

220. Crow, E. L., F. A. Davis, and M. W. Maxfield, *Statistics Manual*, Dover Publications, Inc., New York, 1960.

221. Spackman, D. H., W. H. Stein, and S. Moore, "Automatic recording apparatus for use in the chromatography of amino acids," Analytical Chemistry, 30, 1190 (1958).

222. Spackman, D. H., W. H. Stein, and S. Moore, "Chromatography of amino acids on sulfonated polystyrene resins," Analytical Chemistry, 30, 1185 (1958).

223. Kahn, L., and C. Wayman, "Amino acids in sewage and sewage effluents," Jour. Water Pollution Control Federation, 36, 1368 (1964).

224. Notes on Water Pollution, No. 70, Water Research Centre, Stevenage Laboratory, Stevenage, Herts. (Sept. 1975).

225. A Textbook of Clinical Pathology, S. E. Miller, ed., Williams and Wilkins, Baltimore, Md., 1955.

226. O'Shea, J., and R. L. Bunch, "Uric acid as a pollution indicator," Jour. Water Pollution Control Federation, 37, 1444 (1965).

227. Dunstan, W. M., and D. W. Menzel, "Continuous cultures of natural populations of phytoplankton in dilute treated sewage effluent," Limnology and Oceanography, 16, 623 (1971).

228. Middlebrooks, E. J., D. B. Porcella, E. A. Pearson, P. H. McGauhey, and G. A. Rohlich, "Biostimulation and algal growth kinetics of wastewater," Jour. Water Pollution Control Federation, 43, 454 (1971).

229. Hanson, A. M., and G. F. Lee, "Forms of organic nitrogen in domestic wastewater," Jour. Water Pollution Control Federation, 43, 2271 (1971).

230. Hanson, A. M., and T. F. Flynn, Jr., "Nitrogen compounds in sewage," Proc. 19th Ind. Waste Conference, Purdue Univ., Lafayette, Indiana, 1965.

231. Painter, H. A., and M. Viney, "Composition of a domestic sewage," Jour. of Biochemical and Microbiological Technology and Engineering, 1, 143 (1959).

232. Schlucter, A., "Nitrate interference in total Kjeldahl nitrogen determinations and its removal by anion exchange," U.S.E.P.A. Report 600/7-77-017 (1977).

APPENDIX A

STATISTICAL CALCULATIONS

CALCULATION OF THE COMBINED STANDARD DEVIATIONS SHOWN IN TABLE 7

Before combining the standard deviations from different sets of samples, it must first be determined that all samples are from the same sample population. This can be done by using the variance ratio test (or F-test) described by Moroney [185] at the 5-percent level of significance. For the SCOD analysis:

$$F = \frac{(.50)^2}{(.32)^2} = 2.44$$

$$F(7,7) = 3.79$$

Since $2.44 < 3.79$, the two sets of samples are from the same sample population and their standard deviations, s , may be combined by use of the following equation:

$$s^2 = \frac{(n_1-1)\ s_1^s + (n_2-1)\ s_2^2 + \ldots\ (n_i-1)\ s_i^2}{n_1 + n_2 + \ldots\ n_i - i}$$

where n represents the number of replicate analyses of a sample, i represents the number of sample sets, and s_i represents the standard deviation of a given sample set.

Thus, for the SON analysis:

$$s^2 = \frac{(6-1)(0.03)^2 + (9-1)(0.03)^2 + (4-1)(0.03)^2 + (8-1)(0.03)^2}{6 + 9 + 4 + 8 - 4}$$

$$s^2 = 0.0009$$

$$s = 0.03$$

For the SCOD analysis:

$$s^2 = \frac{(8-1)(0.50)^2 + (8-1)(0.32)^2}{8 + 8 - 2}$$

$$s^2 = 0.1762$$

$$s = 0.42$$

ESTIMATED ERROR OF THE SON AND SCOD ANALYSES

The estimated error of an analysis may be calculated by determining the inherent error in each step of the analysis and then propagating the errors. The errors associated with the SON analysis result from the individual inherent errors in the use of glassware and instruments. The errors listed for SON and SCOD analyses were taken from the manufacturers' specifications, the error printed on the glassware, or from Ref. 187. For the Kjeldahl SON analysis the errors are:

		Error	Relative Error
A.	spectorphotometer reading	0.1% T	± 0.010
B.	volumetric flask (500 ml)	± 0.2 ml	± 0.00040
C.	graduated cylinder (350 ml)	± 1 ml	± 0.00286
D.	Nessler tube (50 ml)	± 0.05 ml	± 0.0010
E.	Nessler tube + Nessler's reagent (52 ml)	± 0.07 ml	± 0.0014

The error of the spectrophotometer given by the manufacturer (Bausch and Lomg) is 0.1% T. The minimum detectable change in concentration (MDC) for such an instrument is given by:

$$\mathrm{MDC} = \frac{2^{1/2}(0.001)}{T \ln T}$$

The transmittance (T) for the least concentrated SON samples was always less than 0.851 (absorbance = 0.070), giving

$$\mathrm{MDC} = \frac{2^{1/2}(0.001)}{(0.851)\ \ln(0.851)}$$

$$= 0.010$$

The estimated error (E) in the Kjeldahl SON analysis resulting from the inherent errors in the measurements above is calculated as follows:

$$E^2 = (0.010)^2 + (0.0004)^2 + (0.00286)^2 + (0.0014)^2$$

$$E^2 = 0.00011$$

$$E = 0.0105$$

$$E = 0.05\%$$

For the Technicon SON analysis, the inherent errors were:

	Error	Relative Error
Digestion tubes (75 ml)	± 0.08 ml	± 0.0011
Pipetting of sample (20 ml)	± 0.02 ml	± 0.0010
Instrument error	± 1% full-scale	± 0.01

Detection of the colorimeter-recorder system of the technicon was given by the manufacturer as ± 1% of full-scale deflection. For low-level SON determinations, full scale was adjusted to equal 1 mg/l SON, making the relative error ± 0.01. Since calculation of the SON concentration involved subtracting both a baseline reading and a sample blank reading, propagation of individual inherent errors results in

$$E^2 = \left[(.01)^2 + (.01)^2 + (0.1)^2 \right] + (.001)^2 + (.0011)^2$$

$$E = 1.74\%$$

For the SCOD analysis the inherent errors are:

		Error	Relative Error
A.	Pipetting of sample (20 ml)	± 0.02 ml	0.001
B.	Pipetting of dichromate (10 ml)	± 0.02 ml	0.002
C.	Titration of sample	± 0.05 ml	-
D.	Titration of blank	± 0.05 ml	-
E.	Titration of standard (25 ml)	± 0.05 ml	0.002

Since calculation of SCOD involves subtration of the sample titration volume from the blank titration volume, the resultant difference has an error of

$$[(0.05)^2 + (0.05)^2]^{1/2} = \pm\ 0.071 \text{ ml}$$

The relative error of this difference is dependent upon the SCOD of the sample. For a sample with a SCOD of 20 mg/l, the difference is 5 ml, and

$$E^2 = \left(\frac{0.071}{5}\right)^2 + (.001)^2 + (.002)^2 + (.002)^2$$

$$E^2 = .000211$$

$$E = .0145$$

$$E = (.0145)(20) = 0.29 \text{ mg/l}$$

For duplicate SCOD analyses, as run during this study,

$$E = \frac{0.29}{(2)^{1/2}} = 0.21 \text{ mg/l}$$

The estimated errors of the SON and SCOD analyses are less than the standard deviations of these analyses, indicating that the deviations shown are reasonable.

CALCULATION OF CONFIDENCE LEVELS

The confidence level (CI) for a given measurement is equal to the product of the standard deviation and students' t divided by the square root of the number of observations [186].

Students' t is a function of the degree of confidence (such as 68, 95, or 99 percent confidence) and the degrees of freedom in calculating the standard deviation. The number of degrees of freedom is given by the total number of samples minus the number of sample sets. For the SON and SCOD analyses, as shown in Table 7, the degrees of freedom are 23 and 14, respectively.

For the SON analysis, in the case of a single observation,

$$\begin{aligned} CI(95\%) &= ts/(1)^{1/2} \\ &= 2.064(0.03) \\ &= 0.06 \\ CI(99\%) &= 2.797(0.03) \\ &= 0.08 \end{aligned}$$

For the SCOD analysis, in the case of a duplicate observation:

$$\begin{aligned} CI(95\%) &= ts/(2)^{1/2} \\ &= \frac{2.145(0.42)}{1.414} \\ &= 0.6 \\ CI(99\%) &= \frac{2.977(0.42)}{1.414} \\ &= 0.9 \end{aligned}$$

The discussion of the results of the experiments described in Section 7 is largely concerned with the arithmetic difference between two measurements, and in many cases this difference is quite small. The standard deviation of the difference between two numbers equals the square root of the sum of the squares of the individual standard deviations. Thus the confidence level for the difference between two numbers equals the confidence level for a single observation multiplied by the square root of 2. The confidence levels for the difference between two analyses are presented in Table A-1, along with those for single analyses.

The results of many of the experiments are expressed in terms of percent removal. Calculation of percent removal involves division of the difference between two measurements by the concentration present in the untreated sample. The standard deviation of percent removal depends upon the relative standard deviations of two numbers, and thus cannot be calculated precisely without knowing the particular numbers involved. An example of such a calculation is as follows:

Consider a case in which the initial SON concentration is 1.00 mg/l and two treated samples contain 0.40 and 0.25 mg/l of SON. The difference between these samples is 0.15 ± .04 mg/l (or 0.15 ± 0.12 mg/l with 99% confidence).

The difference in percent removal (R) is given by:

$$R = \frac{0.40 - 0.25}{1.00} (100) = 15\%$$

The relative standard deviation of R, S_R, is given by

$$\begin{aligned} S_R{}^2 &= \left(\frac{0.04}{0.15}\right)^2 + \frac{0.03}{1.00}{}^2 \\ &= 0.0793 \\ S_R &= 0.282 \end{aligned}$$

TABLE A-1. CONFIDENCE LEVELS FOR SON AND SCOD ANALYSES

	Analysis		Confidence Level, Percent
	SON	SCOD*	
Value for a single analysis, mg/l:	± 0.03	± 0.3	67
	± 0.06	± 0.6	95
	± 0.08	± 0.9	99
Value for the difference between two analyses, mg/l:	± 0.04	± 0.4	67
	± 0.08	± 0.9	95
	± 0.11	± 1.3	99

*Duplicate samples.

In this example the relative standard deviation of the initial concentration is negligible and S_R is approximately equal to the relative standard deviation of the difference:

$$\frac{0.042}{0.15} = 0.280 \simeq 0.282$$

This will be true whenever the difference is small compared to the initial concentration, i.e., for small values of percent removal.

SIGNIFICANCE TESTING FOR DIFFERENCES BETWEEN TWO ANALYSES

Students' t test may be used to test the hypothesis that two means do not differ significantly. The quantity t is defined as the difference between two means divided by its standard deviation [186]. To be significant, a difference must exceed the produce of Students' t and its standard deviation, the confidence level being determined by the confidence level of t. The confidence levels shown in Table A-1 for the difference between two analyses are equal to the product of Students' t and the standard deviation; thus, differences must exceed the values shown in Table A-1 to be considered significant at a given confidence level. For example, the difference between two SON analyses is significant with 95% confidence if it exceeds 0.08 mg/l.

In this study, the data are frequently expressed in terms of percent removal. The difference in percent removal which is significant is dependent upon the initial concentration of SON or SCOD of the sample, since it equals the appropriate value from Table A-1 multiplied by 100 and divided by the initial concentration. Significant differences in percent removal as a function of initial SON or SCOD concentration and confidence level are presented in Table A-2.

SIGNIFICANCE TESTING FOR DIFFERENCES BETWEEN SETS OF ANALYSES

In some instances, it is desirable to compare the difference between two sets of analyses run on a number of different samples. The sample variation may be quite large in some cases, concealing differences in the data. This necessitates a pair-wise comparison of the data points, and the application of Students' t test to the difference between means of correlated observations as described by Edwards [188].

For example, in Table 23, one might wish to know whether ferric chloride removes significantly more SCOD than does lime. Since there is considerable sample variation, only those samples may be considered for which both coagulants were used (in this case, Samples 1 and 4 through 7). The data to be used for the sample calculation is shown in Table A-3, and was taken from Table 23.

The significance test is as follows [188):

n = the number of paired observations

TABLE A-2. SIGNIFICANT DIFFERENCES IN PERCENT REMOVAL

	Difference in Percent Removal to Be Exceeded to Constitute a Significant Difference		
Concentration, mg/l	67% CI	95% CI	99% CI
Initial SON:			
0.90	4	9	12
1.00	4	8	11
1.10	4	7	10
1.20	3	7	9
1.30	3	6	8
1.40	3	6	8
1.50	3	5	7
1.60	3	5	7
1.70	2	5	6
Initial SCOD:			
20.0	2	5	7
22.5	2	4	6
25.0	2	4	5
27.5	1	3	5
30.0	1	3	4

TABLE A-3. REMOVAL OF SCOD BY FERRIC CHLORIDE AND LIME

Sample	Percent SCOD Removed by $FeCl_3$	Percent SCOD Removed by CaO	D	D^2
1	19	21	-2	4
2	39	36	3	9
3	29	22	7	49
4	27	29	-2	4
5	31	14	17	289
n = 5	$\bar{x}_1 = 29.0$	$\bar{x}_2 = 24.4$	$\Sigma = 23$	$\Sigma = 355$

S(D) = the standard deviation of the differences between the paired observations

$\overline{x}_1$ = the average of the first set of observations

$\overline{x}_2$ = the average of the second set of observations

D = the difference between paired observations

$s(\overline{x}_1 - \overline{x}_2)$ = the standard error of the difference between two means when observations are paired.

$$S(D) = \left[\frac{\Sigma(D^2) - (\Sigma d)^2/n}{n - 1}\right]^{1/2} = \left[\frac{355 - (23)^2/5}{4}\right]^{1/2}$$

$$= 7.89$$

$$s(\overline{x}_1 - \overline{x}_2) = \frac{S(D)}{n^{1/2}} = \frac{7.89}{5^{1/2}} = 3.53$$

$$t = \frac{\overline{x}_1 - \overline{x}_2}{s(\overline{x}_1 - \overline{x}_2)} = \frac{29.0 - 24.4}{3.53} = 1.30$$

For a table of Students' t , we find

$$t_{.95,4} = 2.776$$

Since 2.776 > 1.19, the difference between the means is not significant (at a 95% level of confidence).

STATISTICAL METHODS USED IN SECTION 8

Precision and Accuracy of SON and SCOD Analyses

Data in Tables A-4 to A-7 show the precision and accuracy of the SON and SCOD analyses used for the studies described in Section 8.

Confidence Levels for SON and SCOD Analyses

Confidence levels for low-level SON (< 1.3 mg/l) and SCOD (< 25 mg/l) analyses were calculated as described under Calculation of Confidence Levels.

Confidence levels for high-level SON and SCOD values were estimated by noting that relative deviations, sample set standard deviations divided by the average concentration of the set, were essentially constant. For example, from Table A-7:

TABLE A-4. PRECISION AND ACCURACY OF LOW-LEVEL KJELDAHL SON ANALYSES

Type of Sample	Number of Replicates	Average SON mg/1	Standard[a] Deviation mg/1	Reference
0.05 mg/1 Norleucine standard	4	0.05	0.01	This study
0.10 mg/1 Norleucine standard plus 40 mg/1 NH_3-N	5	0.12	0.02	This study
40 mg/1 NH_3-N	4	0.01	0.02	This study
0.91 mg/1 E.P.A. standard[b]	5	0.89	0.03	This study
1.06 mg/1 Norleucine standard plus 20 mg/1 NH_3-N	5	1.07	0.03	This study
Stanford Tap Water	5	0.07	0.03	This study
Stanford Tap Water	4	0.04	0.02	This study
Palo Alto Secondary Effluent	3	1.16	0.03	This study
Palo Alto Secondary Effluent	3	1.11	0.03	This study
Palo Alto Secondary Effluent	3	1.17	0.04	This study
AS Culture 2 Effluent	3	0.24	0.02	This study
Palo Alto Secondary Effluent	6	0.96	0.04	Keller[c]
Palo Alto Secondary Effluent	9	1.08	0.03	Keller
Activated Carbon Effluent	4	0.19	0.04	Keller
Activated Carbon Effluent	8	0.38	0.03	Keller

All samples were double-distilled.

[a] Combined standard deviation = ±0.03 mg/1 as determined by the method described in Appendix B, items 3 and 4.

[b] Prepared standard provided by E.P.A. Analytical Quality Control Laboratory, Cincinnati, Ohio.

[c] Data provided by John V. Keller, Research Assistant, Stanford University.

TABLE A-5. PRECISION AND ACCURACY OF TECHNICON SON ANALYSIS

Type of Sample	Number of Replicates	Average SON mg/l	Standard[a] Deviation mg/l	Reference
3.0 mg/l Norleucine standard	8	2.97	0.06	This study
1.0 mg/l Norleucine standard	4	1.00	0.04	"
0.2 mg/l Norleucine standard	4	0.20	0.05	"
4.0 mg/l Norleucine standard plus 20 mg/l NH_3-N	8	4.14	0.12	"
3.0 mg/l Norleucine standard plus 20 mg/l NH_3-N	8	3.00	0.06	"
2.0 mg/l Norleucine standard plus 20 mg/l NH_3-N	8	2.06	0.06	"
1.0 mg/l Norleucine standard plus 20 mg/l NH_3-N	4	1.03	0.10	"
0.2 mg/l Norleucine standard plus 20 mg/l NH_3-N	4	0.34	0.08	"
20 mg/l NH_3-N	4	0.08	0.05	"
20 mg/l NH_3-N	8	0.10	0.06	"
Palo Alto AS Effluent	3	1.08	0.08	"
Palo Alto AS Effluent	3	1.22	0.08	[129]
Palo Alto AS Effluent	4	1.17	0.07	[129]
San Jose/Santa Clara AS Effluent	2	1.69	0.06	This study
AS Culture 1 Effluent	10	0.35	0.07	"
Palo Alto Primary Effluent	8	4.12	0.11	"
Palo Alto Primary Effluent	8	4.14	0.12	"

[a] Combined standard deviation = ±0.08 mg/l.

TABLE A-6. PRECISION OF LOW-LEVEL SCOD ANALYSIS

Type of Sample	Number of Replicates	Average SCOD mg/l	Standard[a] Deviation mg/l
Stanford Tap Water	3	2.8	1.3
Stanford Tap Water	6	2.5	0.4
Stanford Tap Water	3	2.4	0.4
Palo Alto Secondary Effluent	3	23.4	1.1
Palo Alto Secondary Effluent	3	20.6	0.8
Palo Alto Secondary Effluent	3	23.6	1.1
Palo Alto Secondary Effluent	3	25.9	0.4
AS Culture 1 Effluent	3	24.3	1.1
AS Culture 2 Effluent	3	16.8	0.8

All data from this study.

[a] Combined standard deviation = ±0.8 mg/l.

TABLE A-7. PRECISION OF KJELDAHL SON AND SCOD ANALYSES AT HIGH CONCENTRATIONS

Type of Sample	Analysis	Number of Replicates	Average Concentration (mg/l)	Standard Deviation (mg/l)	Combined Standard Deviation (mg/l)
Palo Alto Primary Effluent	SON	3	5.31	0.22	0.17
"	"	3	4.19	0.13	
"	"	3	4.47	0.16	
Palo Alto Raw Wastewater	"	3	5.50	0.17	
AS Culture 2 Effluent	SON	6	2.03	0.06	0.06
Palo Alto Primary Effluent	SCOD	3	89.5	2.9	3.5
"	"	3	97.6	1.5	
"	"	3	103.3	5.7	
Palo Alto Raw Wastewater	"	3	92.7	2.2	
AS Culture 2 Effluent	SCOD	6	44.5	1.8	1.8

Sample	Average SON (mg/l)	Standard Deviation (mg/l)	Relative Deviation (mg/l)
Primary effluent	5.31	0.22	0.04
Primary effluent	4.19	0.13	0.03
Primary effluent	4.47	0.16	0.04
Raw wastewater	5.50	0.17	0.03
AS Culture 1	2.03	0.06	0.03

The average relative deviation is 0.03 ± 0.0006, compared to the combined standard deviation of ± 0.03 mg/l for low-level SON (< 1.3 mg/l). Since high-level SON determination involves multiplying the concentration actually measured by the dilution factor used to bring the sample into the 0.8-1.3 mg/l range, it was felt that estimating the high-level standard deviation as shown below was reasonable:

$$s(\mathrm{SON} > 1.3\ \mathrm{mg/l}) = \mathrm{SON}(0.03)$$

Similarly, the standard deviation of high level SCOD values (> 25 mg/l) was found to vary as SCOD increased, the average relative deviation for the samples listed in Table A-7 was 0.03 ± 0.02. For purposes of this report, high-level SCOD standard deviation was taken to be

$$s(\mathrm{SCOD} > 25\ \mathrm{mg/l}) = \mathrm{SCOD}(0.03)$$

Results from these studies in many cases were compared by noting arithmic differences between two analyses. The standard deviation of such a difference is the square root of the sum of the squares of the individual standard deviations. Confidence levels for differences can then be calculated by multiplying the confidence level for a single analysis by the square root of 2. Confidence levels for single analyses and for differences between two analyses are presented in Table A-8.

Confidence Levels for Differences Involving SON_{oc} and SCOD_{oc}

For some results in Section 8, a statistical comparison between measured SON and initially calculated SON (SON_{oc}, Eq. 7) was required. SON_{oc} is the weighted sum of two measured SON values, making the standard deviation of differences involving SON_{oc}:

$$s = \sqrt{(.03)^2 + (.03)^2 + (.03)^2}$$

Differences involving SCOD_{oc} are similarly defined. Appropriate confidence levels for these differences are listed in Table A-9.

TABLE A-8. CONFIDENCE LEVELS FOR SON AND SCOD ANALYSES

	Value for single analyses (mg/1)		Value for difference between two analyses (mg/1)	
	95% CI	99% CI	95% CI	99% CI
Kjeldahl SON (< 1.30 mg/1)	±0.06	±0.08	±0.08	±0.11
Kjeldahl SON (> 1.3 mg/1)	±0.06(SCOD)	±0.08(SCOD)	±.008(SCOD)	±0.11(SCOD)
Technicon SON	±0.16	±0.21	±0.23	±0.30
SCOD (< 25 mg/1)	±1.6	±2.3	±2.4	±3.3
SCOD (> 25 mg/1)	±0.06(SCOD)	±0.08(SCOD)	±0.09(SCOD)	±0.12(SCOD)

TABLE A-9. CONFIDENCE LEVELS FOR DIFFERENCES INVOLVING SON_{oc} AND SCOD_{oc}

Analysis	95% CI	99% CI
Kjeldahl SON (< 1.3 mg/1)	±0.10 mg/1	±0.14 mg/1
Technicon SON	±0.28 mg	±0.37 mg/1
SCOD (< 25 mg/1)	±2.9 mg/1	±3.9 mg/1

STATISTICAL METHODS USED FOR MOLECULAR WEIGHT DISTRIBUTION STUDIES

1. Negligible error in measuring volumes was assumed.

2. Values for organic mass standard deviations for concentrated samples were calculated from:

$$s_m = (s)\ (V_o)\ \left(\frac{V_a}{V_c}\right)$$

where

s_m = mass standard deviation, µg or mg;

V_o = volume of original sample concentrated, liters;

V_a = volume applied to Sephadex column, ml;

V_c = volume of concentrate, ml; and
s = standard deviation of analysis, mg/l.

Values used for s are listed below (from Statistical Methods used in Section 8).

Kjeldahl SON -	raw wastewater	$s = \pm 0.17$ mg/l
	primary effluent	$s = \pm 0.16$ mg/l
	AS effluent	$s = \pm 0.03$ mg/l
	AS Culture 2 eff.	$s = \pm 0.06$ mg/l
SCOD -	raw wastewater	$s = \pm 2.8$ mg/l
	primary effluent	$s = \pm 2.7$ mg/l
	AS effluent	$s = \pm 0.8$ mg/l
	AS Culture 2 eff.	$s = \pm 1.3$ mg/l

Using these values for s, appropriate values for s_m are:

Sample	s_m(SON)	s_m(SCOD)
Raw wastewater	± 17 µg	± .28 mg
Primary effluent	± 16 µg	± .27 mg
AS effluent	± 7 µg	± .18 mg
AS Culture 2 effluent	± 11 µg	± .23 mg

3. Estimates of 95 percent confidence limits for mass measurements were made from the following:

$$\mu = \overline{X}_m \pm t \frac{s_m}{\sqrt{n}}$$

where

μ = estimated population mean, µg or mg;
$\overline{X}_m$ = mass average of replicate samples, µg or mg;
s_m = as determined in 2;
n = 2; and
t = t statistic.

For each wastewater studied:

Sample	SON (μg)	SCOD (mg)
Raw wastewater	$\overline{X} \pm 23$	$\overline{X} \pm 0.41$
Primary effluent	$\overline{X} \pm 22$	$\overline{X} \pm 0.40$
AS effluent	$\overline{X} \pm 10$	$\overline{X} \pm 0.34$
AS Culture 2 effluent	$\overline{X} \pm 16$	$\overline{X} \pm 0.34$

These confidence limits were reported in Table 82 and were used to calculate confidence limits for percent recoveries.

4. The following procedure was used to calculate 95 percent confidence limits for SON and SCOD mass contained in the five MW fractions (Tables 83 and 84).

   The SON and SCOD of the eluant were subtracted from the measured SON and SCOD of each MW fraction. The standard deviation of such a difference is the square root of the sum of the squares of the individual standard deviations [218], and for the Technicon SON and low-level SCOD analyses are 0.11 and 1.1 mg/l, respectively. Values for 95 percent confidence limits were then estimated from the following

$$\mu_{MW} = V_f(\overline{X}_{MW} \quad t\ s_d/\sqrt{n}\ )$$

In which

μ_{MW} = estimated population mean, μg or mg;

V_f = volume collected for a particular MW fraction, liter;

$\overline{X}_{MW}$ = average SON or SCOD concentration for a particular MW fraction, mg/l;

t = t statistic for SON or SCOD analysis;

s_d = standard deviation as described above, mg/l; and

$n = 2$ (replicate analyses).

For Technicon SON and low-level SCOD analyses,

$$\mu_{MW}(\text{SON}) = V_f(\overline{X}_{MW} \pm 0.16)$$

$$\mu_{MW}(\text{SCOD}) = V_f(\overline{X}_{MW} \pm 1.7)$$

5. Confidence limits (95 percent level) for percent recoveries were estimated using [186]:

$$\left(\frac{s_R}{R}\right)^2 = \left(\frac{s_A}{A}\right)^2 + \left(\frac{s_B}{B}\right)^2$$

where

$R = A/B$;

s_R = standard deviation of the ratio A/B;

s_A, s_B = standard deviations for A and B, respectively
B = initial concentration (SON or SCOD, mg/l); and
A = final concentration (SON or SCOD, mg/l).

To estimate percent recoveries from original sample or from organic mass applied to the Sephadex column, s_B was calculated as in 3 for a 95 percent level of significance. When estimating total recovery through the Sephadex column, s_A was calculated by summing values from 4 as shown below [218]:

$$s_A = \pm \sqrt{\sum_i \left(t \frac{s_d}{2} \right)^2}$$

These values are reported in Tables 83 and 84.

For calculating 95 percent confidence limits for data presented in Figures 34 and 37, values for s_A were those calculated in (4).

The estimated 95 percent confidence limits were then reported as

$$A/B \pm s_R$$

for the cases described above.

6. For differences in percent of original SON or SCOD mass contained in two MW fractions (Figures 34 to 37) to be significant at a 95 percent confidence level, they must be greater than [218]

$$\pm t \frac{s_d}{\sqrt{n}}$$

where s_d is the standard deviation of the difference, t is the t statistic, and n is one. s_d can be determined from s_r values calculated in 5. Since these values for s_r include the t statistic, the difference required for 95 percent confidence becomes

$$\pm \sqrt{{s_{R1}}^2 + {s_{R2}}^2}$$

where s_{R1} and s_{R2} are s_R values for the two MW fractions being compared.

7. Statistical Analyses of SON/SCOD Ratios

   a. Confidence levels (95 percent CI) for SON/SCOD ratios were calculated using standard deviations, t statistics, and the equation listed below [186]:

$$s_R = \pm \frac{SON}{SCOD} \sqrt{\frac{t_{SON} \cdot s_{SON}}{\sqrt{n} \cdot SON}^2 + \frac{t_{SCOD} \cdot s_{SCOD}}{\sqrt{n} \cdot SCOD}^2}$$

where

t_{SON}, t_{SCOD} = t-statistics for SON and SCOD;
n = number of analyses;
s_{SON}, s_{SCOD} = standard deviations for SON and SCOD analyses
SON = measured SON;
SCOD = measured SCOD; and
s_R = 95% CI.

b. For differences in SON/SCOD ratios to be significant, they must be greater than:

$$\pm \sqrt{s_{R1}^2 + s_{R2}^2}$$

where s_{R1} and s_{R2} are the 95% CI values for the two ratios being compared.

APPENDIX B

NITRATE INTERFERENCE WITH KJELDAHL SON ANALYSIS

High levels of nitrate nitrogen were found to interfere with Kjeldahl analysis for SON. The problem was discovered during a biodegradation study with Palo Alto activated-sludge effluent in which SON decreased from 1.45 mg/l to a value calculated to be less than zero after 50 days of degradation, an unreasonable result based on previous biodegradation results. Preliminary experiments indicated high nitrate concentrations caused the abnormal SON decrease. The following is a brief discussion of factors found to affect the interference, the mechanisms involved, and methods attempted to eliminate the interference.

FACTORS AFFECTING THE INTERFERENCE

Tables B.1 and B.2 contain data describing the magnitude in SON reduction caused by various concentrations of NO_3^--N. It appears the critical NO_3^--N concentration for interference is between 6 and 10 mg/l, and may vary for different samples. The magnitude of the interference was non-reproducible at high NO_3^--N values; SON recoveries ranged from 11-57 percent (5 samples) at 40 mg/l NO_3^--N. The presence and concentration of NO_2^--N (0-40 mg/l), alkalinity (0-111 mg/l as $CaCO_3$), and Cl^- (0-2000 mg/l) did not produce nor prevent the interference. Addition of reducing organics such as glucose was found to "produce" SON by reducing NO_3^- to NH_3 during digestion, the NH_3-N is then measured as SON; thus creation of a positive interference by certain organics was also found to be possible.

MECHANISMS INVOLVED

The interference occurred during the digestion step, and involved a reaction between NH_3 and NO_3^- resulting in the disappearance of all, or a portion of the NH_3. A recent study by Schlueter[232] which was instigated as a result of our finding has shown that the probable mechanism is as follows:

$$NH_4^+ + NO_3^- = N_2O + 2H_2O$$

The nitrous oxide excapes in the digestion gas as confirmed by infrared analysis of the collected gases [232].

TABLE B.1. EFFECT OF NO_3^--N CONCENTRATION ON SON RECOVERY FROM A BIOLOGICALLY TREATED WASTEWATER*

Added NO_3^--N (mg/1)	Measured SON (mg/1)	Percent SON Recovery
1.4	0.55	100 (assumed)
6.4	0.53	96
11.4	0.42	76
21.4	0.41	75
31.4	0.23	51
41.4	0.19	35

*Effluent from a symbiotic biological process (grass-bacteria) treating agricultural return water; see REf. 76 for description.

TABLE B.2. EFFECT OF NO_3^--N CONCENTRATION ON SON RECOVERY FROM PALO ALTO ACTIVATED-SLUDGE EFFLUENT

NO_3-N (mg/1)	SON (mg/1)	Percent Recovery
0 (assumed)	1.23	100 (assumed)
5	1.23	100
10	1.22	99
15	0.86	70
20	1.14	93

METHODS ATTEMPTED TO ELIMINATE THE INTERFERENCE

A simple, rapid chemical method was sought, one that would quantitatively reduce the NO_3^- to non-interfering NO_2^- or NH_3, or, alternatively, a modified digestion technique that would not produce the interference. Digestion with catalysts other than HgO [3], with permaganate, hydrogen peroxide, and mixtures of these compounds with sulfuric acid did not eliminate the interference. This observation should have been expected since the interference reaction involved (conversion to N_2O) is catalyzed by high-temperature acid conditions similar to those used for the standard and modified Kjeldahl digestions [232]. A wide range of reducing compounds (Table B.3) were tried and none eliminated the interference.

Schlueter [232] reported that removal of NO_3^- by anionic exchange resulted in 100 percent recovery of the SON compound alanine. However, results presented in this research showed anionic resins remove a significant portion of treated and untreated wastewater SON, and thus may have limited application in eliminating the NO_3^- interference with these types of samples. Perhaps a selective resin, removing NO_3^- but no wastewater SON, can be found. This warrants further study.

CONCLUSIONS

Nitrate nitrogen interferes with SON analysis by the Kjeldahl method, resulting in decreased SON recovery. During this research, NO_3^--N was monitored at all times, and SON values for samples containing NO_3^--N in excess of 7 mg/l were not reported, or reported as questionable.

TABLE B.3. EFFECT OF COMPOUNDS TRIED FOR ELIMINATION OF NITRATE INTERFERENCE

Glucose	+	Cd S	+	$Na_2S_2O_3$	+	Fe^o	+
Na Acetate	o	FeS	+	Na_2SO_6	+	Cd^o	+
Pthalic Acid	+	SeS_2	+	Na_2SO_3	-	Cu	-
Parafin	+	S^o	+	$NaAsO_2$	-	$FeCl_2$	-
						$CrCl_3$	-

+ increased SON recovery in presence of 40 mg/l NO_3^--N.

- decreased SON recovery in presence of 40 mg/l NO_3^--N.

o no effect on SON recovery in presence of 40 mg/l NO_3^--N.